



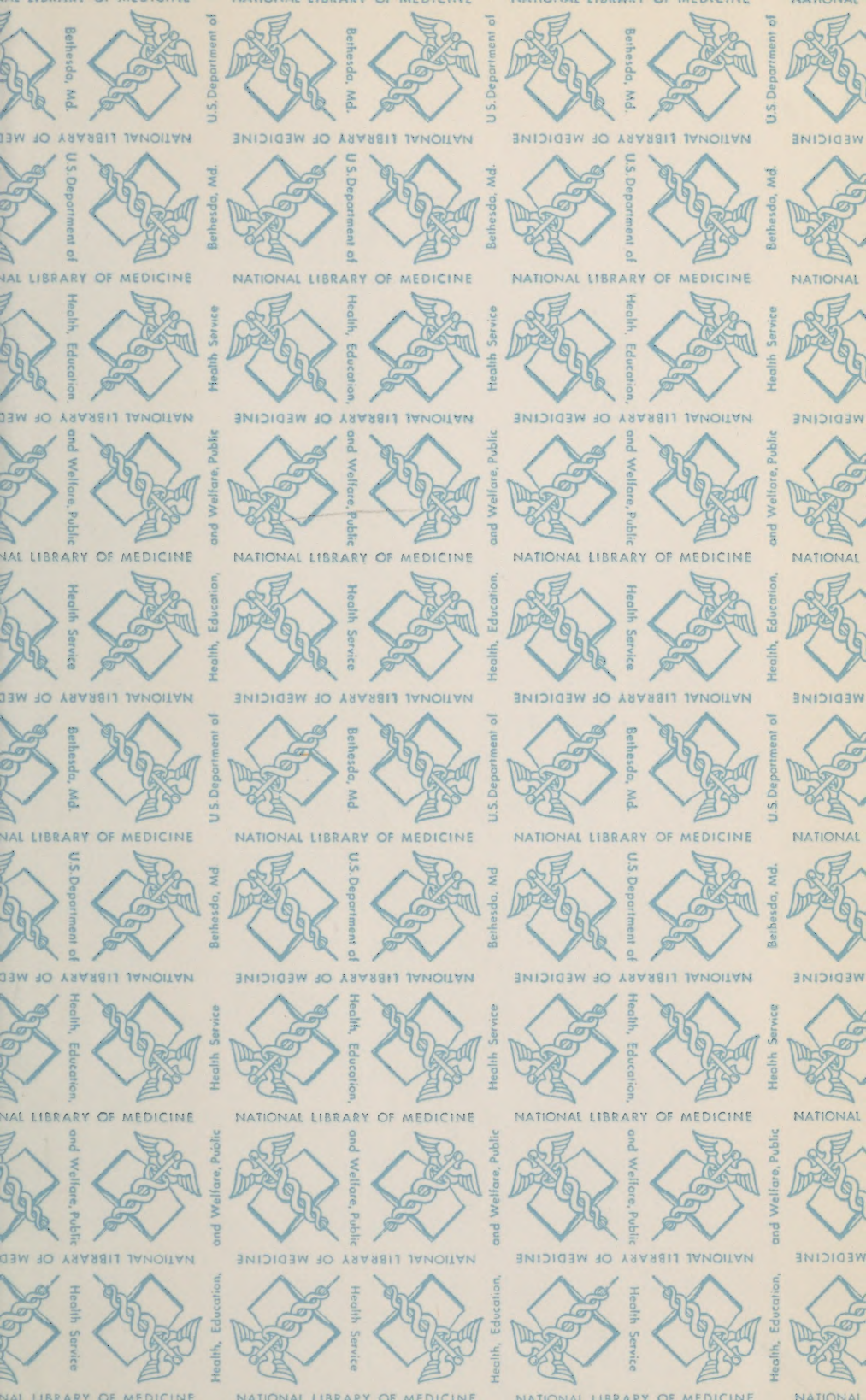
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DIAGNOSTIC METHODS

WEBSTER

DIAGNOSTIC METHODS

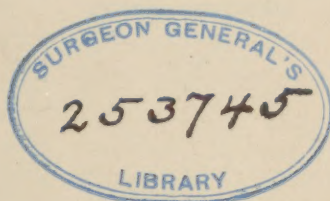
CHEMICAL, BACTERIOLOGICAL AND MICROSCOPICAL

A Text-book for Students and Practitioners

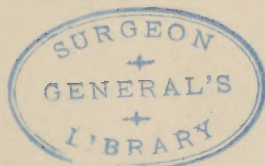
BY

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SEVENTH EDITION, REVISED AND ENLARGED
WITH 37 COLORED PLATES
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TO THE MEMORY
OF
MY FATHER
DR. JOHN RANDOLPH WEBSTER
THIS VOLUME IS
LOVINGLY DEDICATED

PREFACE TO THE SEVENTH EDITION

While only two years have elapsed since the appearance of the last edition of this book, yet there has been considerable research along many lines of Clinical Diagnosis and many new publications have appeared which had been held in abeyance for obvious reasons during the war. The author therefore, has had little difficulty in finding a wealth of new material to add to this edition.

Among these additions will be noted new methods for typing pneumococci in sputum; a discussion of the subject of Bronchial Spirochetosis; Albert's stain for diphtheria bacilli; Fractional Method of Rehfuss for examination of stomach contents; Meltzer-Lyon method for obtaining bile; the brine flotation-loop method of Kofoed and Barber for detecting ova in stools; Bell and Doisy's methods for inorganic phosphates, total and organic phosphorus in urine and blood; Folin's method for amino-acids in urine, Urea Concentration Test of MacLean and de Wesselow; Hektoen's Precipitin Test for semen; Van Slyke's method of determining oxygen capacity and hemoglobin content of blood; Stadie's method for methemoglobin in blood; Van Slyke and Silverman's method for carbon monoxid in blood; Benedict's new method for uric acid in blood; Tisdall's method for inorganic phosphates in blood; Kramer and Tisdall's method for calcium in blood; Whitehorn's method for chlorids in blood; Kolmer's Standardized Quantitative Wassermann Test; Warthin and Starry's method for spirochetes in tissue; Noguchi's new method for proteins in cerebrospinal fluid; Colloidal Benzoin Test of Guillain, Laroche and Lechelle; and an extended discussion and description of the various pathogenic anaerobic organisms which are found in wounds, especially the *Vibrio septique*, *Bacillus oedematiens*; *Bacillus sporogenes* and *Bacillus tetani*. Under this head is included a discussion of *Bacillus botulinus* as a pathogenic anaerobe. Throughout the subject-matter of the text new material has been inserted and references have been revised up to date in order to increase the working value of the book as a reference work.

The author wishes to express his gratification at the continued cordial reception of the work and to thank his reviewers and friends for helpful suggestions. To his publishers he wishes to express his appreciation of their many courtesies during the years in which he has been associated with them. It is hoped that this new edition may be found acceptable to student, practitioner and laboratory worker.

RALPH W. WEBSTER.

25 E. WASHINGTON ST., CHICAGO.

PREFACE TO THE SIXTH EDITION.

During the few years since the appearance of the last edition, original work along all lines has suffered from the absence of many of our associates in the service. However, quite an amount of material, of sufficient importance to warrant inclusion in this edition, has been forthcoming from various sources.

Among these additions will be found quite a number of new methods from the laboratory of Folin such as Folin and Bell's direct nesslerization method for ammonia in urine; Folin and Denis' direct nesslerization method for total N in urine, and their method for lactose in milk; Folin and McEllroy's test for sugar in the urine; Folin and Peck's method for sugar in the urine; Folin and Wright's simplified Kjeldahl method; Folin and Wu's system of blood analysis, which includes methods for creatin and creatinin in blood, non-protein nitrogen in blood, sugar in blood, urea in blood, uric acid in blood, and uric acid in urine; Folin and Youngburg's direct nesslerization method for urea in urine. The subject of functional renal diagnosis has been extensively enlarged and a full discussion of Mosenthal's Test Meal for Renal Function has been included. The section dealing with the reaction of the blood has been entirely rewritten to bring it up to the present day conception of Hydrogen-ion concentration; in this section the following methods have been included, as measures of the reserve alkalinity and the total P_H of the blood: Van Slyke and Cullen's method for CO_2 combining power of the plasma; Van Slyke, Stillman, and Cullen's titration method for plasma bicarbonate; electrometric methods for determination of P_H ; Levy, Rowntree and Marriott's method for hydrogen ion concentration; Marriott's method for alkali reserve. In the discussion of the parasitology of the blood, the subject of Infectious Jaundice has been introduced, a full account of the *Leptospira icterohæmorrhagica* being given. It has been thought advisable to include among the serum reactions for syphilis, the Coagulo Reaction of Hirschfeld and Klinger, as this has great promise. In the section on Clinical Bacteriology, the discussion has now included the Gas Bacillus of Welch, which has assumed considerable importance in the study of wound infections during the war. Throughout the text the subject-matter has been brought thoroughly up to date by reference to the literature of the subjects treated.

The author wishes to express his thanks for the more than cordial way in which the previous editions of this book have been received and to thank his reviewers and friends for words of criticism and praise. It is hoped that this new edition may prove even more acceptable than have former ones.

RALPH W. WEBSTER.

PREFACE TO THE FIRST EDITION.

In the present work the writer has attempted to bring together, for the use of the student and practitioner, the generally accepted facts regarding the various phases of clinical medicine, which may be rather more closely studied by the application of laboratory methods than without their use. It is to be especially emphasized that laboratory work must go hand in hand with the more direct clinical examination of the patient, as the former can be interpreted only in the light of the latter. While it is true that, in some cases, the laboratory findings may be of even more value than those of physical examination, yet it is to be understood that the function of the Clinical Laboratory is, more largely, perhaps, an accessory one, the application of methods of physical diagnosis usually pointing out the way toward a successful solution of the clinical problem by special laboratory methods, which yield numerous confirmatory or differential points not at all clearly defined by the methods of direct clinical examination.

The aim of the author has been to present the direct bearing of the various methods outlined upon the clinical history of the case and to point out the special interpretation of the findings in any given examination. Particular attention has been directed to the selection of methods, both for the simpler clinical and more complex scientific requirements. As no method, no matter how exact it may be or how sound its basic principle, will yield reliable results in the hands of the inexperienced, the writer has paid much attention to the details of such methods and has endeavored to direct the thought of the worker to the possible obstacles to be overcome before he is able properly to perform these examinations and interpret his results.

The writer makes no claim for originality except, possibly, in the matter of arrangement of subject-matter and selection of methods and ideas, which have been established by others after years of earnest research. His endeavor has been to be as catholic as possible in his reading and as selective as his judgment permitted, to the end that the student or practitioner be saved the burden of sifting the wheat from the chaff.

Numerous text-books, monographs, and special articles have been freely used in the preparation of the text, the writer attempting in each case to give due credit for such reference. It is possible that some direct use of material has been made which has not received deserved recognition. If so, the writer here acknowledges his indebtedness to them as well as to those whom he has directly quoted.

It has seemed desirable to omit extensive reference to the literature, as a bibliography to be of working value must be much more extensive and com-

plete than is possible within the scope of this book. The writer has, however, inserted at the end of each chapter a list of the more important larger works which he has found useful in correlating the general subjects included in the several sections.

In conclusion, the writer wishes to express his deep obligation to his colleagues for their many valuable suggestions as to the subject-matter of the text: to Prof. W. S. Haines for assistance in revising the section on Urine; to Dr. J. M. Washburn for many additions and revisions of the section on Blood; to Dr. O. J. West for many practical points throughout the whole work; to Dr. W. A. Pusey for photographs of Blastomycetes under various conditions; to Dr. Brown Pusey for slides showing various organisms in the conjunctival exudates; and to Dr. N. Gildersleeve, of the University of Pennsylvania, for illustrations of megalosporon and microsporon. Further, the writer wishes to express his thanks to Miss Hill for the excellent work done by her in preparing the original drawings which appear throughout the work.

RALPH W. WEBSTER.

TABLE OF CONTENTS

CHAPTER I

The Sputum

	PAGE
I. GENERAL CONSIDERATIONS.	I
II. PHYSICAL AND CHEMICAL CHARACTERISTICS	2
Amount.	2
Consistency	2
Reaction	3
Color.	3
Odor	4
Character.	4
Chemical Properties	5
III. MACROSCOPIC EXAMINATION.	6
Cheesy masses.	6
Dittrich's plugs	7
Curschmann's spirals.	7
Fibrinous casts.	8
Concretions	8
Bronchioliths.	8
Pneumoliths.	9
Echinococcus membranes	9
Foreign bodies.	9
IV. MICROSCOPIC EXAMINATION	9
Pus-cells	10
Red blood-cells.	11
Epithelial cells.	11
Elastic tissue	12
Crystals.	13
Bacteria.	14
Saprophytes.	14
Pathogenic types.	18
Tubercle bacillus.	18
Lepra bacillus	24
Smegma bacillus.	25
Timothy bacillus.	25
Pneumococcus.	26
Friedländer's bacillus.	28
Influenza bacillus.	28
Bacillus pertussis.	29
Bacillus typhosus.	29
Staphylococcus and streptococcus pyogenes	29
Bacillus pestis	29
Bacillus anthracis	30
Bacillus mallei.	30
Actinomyces hominis.	30

	PAGE
Animal parasites.	32
Amebæ	32
Flagellates.	32
Cestodes	32
Trematodes	32
V. THE SPUTA IN DISEASE.	33
Pulmonary tuberculosis.	33
Croupous pneumonia.	34
Broncho-pneumonia	34
Acute bronchitis	34
Chronic bronchitis	35
Simple type	35
Putrid type	35
Fibrinous type.	35
Bronchial asthma.	36
Influenza	36
Gangrene of the lung.	36
Abscess of the lung.	36
Perforating empyema.	37
Pneumonoconioses	37
Bronchial Spirochetosis	37

CHAPTER II

Oral, Nasal, Aural and Conjunctival Secretions

I. ORAL SECRETION.	38
General considerations	38
Microscopic examination	39
Pathologic changes.	40
Pharyngomycosis leptothrica.	41
Diphtheria	42
Vincent's angina.	44
Streptococcic sore throat	45
Gonorrheal stomatitis.	45
Thrush	45
Oral endamebiasis	46
II. NASAL SECRETION	48
General considerations.	48
Pathologic changes.	49
Rhinitis.	49
Hay fever.	50
Meningitis.	50
III. AURAL SECRETION	51
General considerations	51
Pathologic changes.	51
IV. CONJUNCTIVAL SECRETIONS	51
General considerations.	51
Pathologic changes.	52
Diphtheritic conjunctivitis.	52
Infectious conjunctivitis.	52
Gonorrheal conjunctivitis	52
Trachoma.	53
Vernal conjunctivitis	53

CHAPTER III

Gastric Contents

	PAGE
I. GENERAL CONSIDERATIONS	54
II. METHODS OF OBTAINING GASTRIC CONTENTS.	56
Stomach-tube	56
Fractional method of Rehfuß	58
Test meals.	60
Ewald meal	61
Boas meal.	61
Riegel meal	61
Fischer meal.	62
Salzer meal	62
Sahli meal.	62
III. MACROSCOPIC EXAMINATION.	64
Amount.	64
Color.	65
Odor	65
Consistency	66
Contents from fasting stomach.	66
Vomitus.	66
Contents after test meals	68
IV. MICROSCOPIC EXAMINATION	68
General.	68
Food remnants.	69
Boas-Oppler bacillus	69
Sarcinæ ventriculi	70
Protozoa	70
Tissue fragments.	70
Crystals.	70
V. CHEMICAL EXAMINATION.	70
General.	70
Total acidity.	71
Free hydrochloric acid	72
Qualitative tests	73
Töpfer's test.	73
Günzburg's test	73
Boas' test.	73
Tropeolin test	74
Quantitative test.	74
Mintz's method	74
Töpfer's method.	75
Amount of free hydrochloric acid.	75
Euchlorhydria.	77
Hypochlorhydria.	77
Ana-chlorhydria	77
Hyperchlorhydria	78
Combined hydrochloric acid.	78
Method of Martius and Lüttke.	78
Method of Töpfer	79
Hydrochloric acid deficit	80
Organic acids.	80
Total organic acids.	80
Lactic acid	81

	PAGE
Uffelmann's test	82
Kelling's test.	82
Strauss' method	82
Butyric acid.	83
Acetic acid.	84
Gastric ferments.	84
Pepsin	84
Qualitative methods	85
Quantitative examination	86
Hammerschlag's method	86
Mett's method.	86
Method of Thomas and Weber.	87
Chymosin	88
Leo's method	88
Riegel's method	88
Lipase	89
Products of protein digestion.	89
Products of carbohydrate digestion.	89
Blood.	90
Gases.	90
Function of the stomach and contents.	91
VI. MOTILITY OF THE STOMACH.	91
Leube's method.	92
Boas' method.	92
Method of Ewald and Sievers	92
Winternitz' test.	93
VII. ABSORPTIVE POWER OF THE STOMACH	93
Potassium iodid test.	93
VIII. INDIRECT EXAMINATION OF THE STOMACH CONTENTS.	94
Günzburg's method.	94
Sahli's desmoid reaction.	94
IX. GASTRIC JUICE IN DISEASE.	95
Hyperchlorhydria.	95
Hypersecretion	96
Achyia gastrica	96
Acute gastritis	97
Chronic gastritis	97
Nervous dyspepsia	97
Ulcer of the stomach	98
Carcinoma of the stomach.	98
Salomon's test	100
Neubauer and Fischers' test	101
Wolff and Junghan's test.	102

CHAPTER IV

The Feces

I. GENERAL CONSIDERATIONS.	105
Normal feces.	105
Diet of Schmidt and Strasburger	106
Diet of Folin.	107
Obtaining intestinal contents.	107
Obtaining bile from duodenum	109
Functions of the intestinal juice.	110
Estimation of intestinal digestion.	111

	PAGE
II. MACROSCOPIC EXAMINATION	III
Method	III
Amount.	II2
Consistency and form.	II3
Odor	II4
Color	II4
Blood.	II6
Mucus.	II9
Pus.	121
Food remnants.	121
Protein residues	123
Fat residues	124
Carbohydrate residues.	125
Biliary constituents.	125
Intestinal sand and concretions.	126
Tissue fragments.	127
III. MICROSCOPIC EXAMINATION	127
Technic	127
Morphological elements	128
Crystals.	128
IV. CHEMICAL EXAMINATION.	129
Reaction.	129
Total solids.	130
Total nitrogen.	130
Fat.	131
Carbohydrates	132
Phenoltetrachlorophthalein test	134
V. BACTERIOLOGY OF THE FECES.	136
Technic	136
Cholera spirillum.	138
Typhoid bacillus	139
Method of Drigalski and Conradi.	139
Method of Kendall and Day.	141
Bacillus of Dysentery	141
Tubercle bacillus	142
VI. PARASITOLOGY OF THE FECES.	142
Technic	143
Protozoa.	144
Rhizopoda.	144
Amebina.	144
Ameba coli.	145
Endameba coli	149
Sporozoa.	149
Coccidium hominis	149
Flagellata	149
Trichomonas intestinalis.	149
Cercomonas hominis.	150
Megastoma entericum.	150
Infusoria.	151
Balantidium coli	151
Entozoa.	151
Platodes.	151
Cestodes.	151
Tæniidæ.	153
Tænia solium.	153

	PAGE
<i>Tænia saginata</i>	153
<i>Tænia cucumerina</i>	154
<i>Tænia nana</i>	154
<i>Tænia diminuta</i>	155
<i>Tænia echinococcus</i>	155
<i>Bothriocephaloidea</i>	156
<i>Bothriocephalus latus</i>	156
<i>Dibothriocephalus cordatus</i>	157
<i>Bothriocephalus</i> sp. <i>Ijima et Kurimoto</i>	157
Trematodes	157
Nematodes	158
Ascaridæ	158
<i>Ascaris lumbricoides</i>	158
<i>Ascaris mystax</i>	159
<i>Oxyuris vermicularis</i>	159
Angiostomidæ	160
<i>Strongyloides intestinalis</i>	160
Trichotrachelidæ	162
<i>Trichiuris trichiura</i>	162
<i>Trichinella spiralis</i>	162
Strongylidæ	164
<i>Uncinaria duodenalis</i>	164
<i>Uncinaria Americana</i>	165
Pseudo-parasites	166

CHAPTER V

Parasites

I. GENERAL CONSIDERATIONS	169
II. TREMATODES	169
Fasciolidæ	170
<i>Fasciola hepatica</i>	170
<i>Fasciolopsis Buski</i>	171
<i>Opisthorchis felineus</i>	172
<i>Opisthorchis sinensis</i>	172
III. NEMATODES	173
<i>Eustrongylus gigas</i>	173
IV. PARASITES OF THE SKIN	174
Arthropoda	174
Arachnoidea	175
<i>Sarcoptes scabiei</i>	175
<i>Demodex folliculorum</i>	175
<i>Leptus autumnalis</i>	175
Insecta	176
Hemiptera	176
<i>Pediculus capitis</i>	176
<i>Pediculus vestimenti</i>	176
<i>Pediculus pubis</i>	176
<i>Cimex lectularius</i>	177
Diptera	178
<i>Pulex irritans</i>	178
<i>Pulex penetrans</i>	178
Vegetable Parasites	178
<i>Achorion Schönleini</i>	178

	PAGE
Trichophyton megalosporon endothrix.	179
Microsporon Audouini.	180
Microsporon furfur	180
Microsporon minutissimum.	182
Blastomycetes	182
Sporothrix Schenckii	184
Negri Bodies.	185

CHAPTER VI

The Urine

I. GENERAL CONSIDERATIONS.	188
Collection and preservation of the urine.	189
II. PHYSICAL PROPERTIES.	190
Quantity.	190
Polyuria.	191
Oliguria	193
Anuria.	193
Appearance.	194
Color	195
Odor	197
Reaction.	198
Folin's method for total acidity.	199
Free mineral and organic acidity	200
Specific gravity.	202
Technic.	203
Rough estimate of total solids	204
Optical activity.	205
III. CHEMICAL PROPERTIES.	206
Normal composition.	206
Total solids and total ash	207
Inorganic constituents.	208
Chlorids.	208
Estimation of the chlorids.	211
Quantitative determination.	211
Arnold-Volhard method	211
Purdy's centrifugal method.	214
Phosphates.	214
Estimation of phosphates	217
Quantitative determination.	218
Uranium method	218
Methods of Bell and Doisy.	221
Purdy's centrifugal method.	222
Sulphur compounds.	223
Preformed sulphates.	223
Ethereal sulphates	224
Neutral sulphur.	225
Estimation of total sulphur.	226
Folin's method.	226
Determination of total sulphates	227
Folin's method.	227
Determination of inorganic sulphates.	227
Purdy's centrifugal method.	228
Carbonates.	228

	PAGE
Sodium and potassium.	228
Calcium and magnesium.	229
Iron.	231
Organic constituents.	231
Nitrogenous bodies.	231
Total nitrogen	231
Kjeldahl's method	235
Folin and Wright's Simplified Kjeldahl method.	237
Folin and Denis' Direct Nesslerization method	238
Urea.	241
Determination of urea.	242
Knop-Hüfner method	243
Doremus ureometer.	243
Folin's method.	245
Mörner-Sjöqvist method.	246
Urease method.	247
Folin and Youngburg's Direct Nesslerization method	249
Ammonia	250
Quantitative determination.	251
Method of Schlösing.	252
Folin's method.	253
Folin and Bell's Direct Nesslerization method	254
Formalin method.	255
Uric acid.	255
Quantitative determination.	259
Folin and Shaffer's method.	259
Salkowski-Ludwig method	260
Method of Rudisch and Kleeberg.	261
Folin and Wu's Colorimetric method	263
Ruhemann's method	264
Purin bases	265
Creatinin.	266
Qualitative tests	268
Weyl's test.	268
Jaffé's test.	269
Quantitative determination.	269
Folin's methods.	269
Creatin	271
Folin's method.	271
Undetermined nitrogen	272
Amino acids	272
Benedict and Murlin's method	273
Folin's method	274
Hippuric acid.	275
Oxyproteic and alloxypoteic acids	275
Allantoin	276
Fatty acids.	276
Oxalic acid.	277
Quantitative determination.	278
Baldwin's method.	278
Ferments.	279
Pepsin.	279
Diastase	279
Lipase	279

	PAGE
Mucin-like bodies	280
Mucin.	280
Nucleo-albumin.	280
Pigments and chromogens	281
Urochrome.	281
Uroerythrin	282
Urobilin	283
Indican	284
Tests for indican	285
Jaffé's test.	285
Obermayer's test	286
Rosenbach's test	287
Quantitative determination.	287
Wang's method.	287
Folin's method.	288
Uroroseinogen	288
Abnormal Composition	289
Proteins.	289
Serum-albumin.	289
Albuminuria	290
Functional.	290
Febrile	293
Traumatic.	293
Hematogenous	294
Toxic	294
Neurotic.	294
With definite renal lesions.	294
Qualitative tests	295
Heat and acid test	296
Heller's nitric acid test	297
Ferrocyanide test.	300
Sulpho-salicylic acid test.	300
Spiegler's test	300
Quantitative methods.	301
Scherer's method	301
Esbach's method	302
Method of Tsuchiya.	302
Purdy's centrifugal method.	303
Removal of albumin.	303
Serum-globulin.	304
Qualitative tests	304
Quantitative method	305
Proteoses	305
Primary proteoses	305
Bence-Jones' protein	305
Secondary proteoses.	308
Tests	308
Bang's method.	309
Clinical significance.	309
Peptone.	310
Hemoglobin	310
Heller's test.	312
Donogany's test	312
Fibrin.	312
Carbohydrates	313

	PAGE
Glucose	313
Glycosuria	314
Qualitative tests	318
Trommer's test	319
Benedict's test	321
Folin and McEllroy's test	321
Fehling's test	322
Haines' tests	322
Almén-Nylander's test	324
Fermentation test	325
Phenyl-hydrazin test	326
Quantitative tests	328
Folin and Peck's method	328
Bang's method	329
Purdy's method	331
Haines' method	332
Polariscopic method	333
Fermentation method	336
Roberts' method	337
Levulose	338
Levulosuria	338
Seliwanoff's test	339
Phenyl-methyl-hydrazin test	339
Pentose	341
Pentosuria	341
Qualitative tests	342
Tollen's reaction	342
Orcin test	343
Bial's test	343
Quantitative test	343
Diphenyl-hydrazin method	344
Cammidge's reaction	344
Lactose	345
Lactosuria	345
Rubner's test	346
Maltose	346
Maltosuria	346
Glycuronic acid	347
Neuberg's quantitative method	350
Acetone bodies	350
Acetone	354
Qualitative tests	354
Legal's test (or Le Nobel's)	354
Lieben's test	354
Gunning's test	355
Frommer's test	355
Quantitative methods	355
Huppert-Messinger method	356
Folin's method	357
Diacetic acid	358
Qualitative tests	358
Gerhardt's test	358
Arnold's test	359
Lipliawsky's test	359
β -oxybutyric acid	359

	PAGE
Hart's test.	360
Quantitative determination.	360
Black's method.	360
Shaffer's method.	361
Abnormal pigments.	362
Blood pigments.	362
Hemoglobin.	362
Hematoporphyrin.	362
Biliary pigments.	363
Qualitative tests.	364
Smith's test.	364
Gmelin's test.	364
Rosembach's test.	365
Nakayama's test.	365
Hammarsten's test.	365
Bile acids.	365
Hay's test.	366
Oliver's test.	366
Melanin.	366
Phenol derivatives.	367
Alkapton.	367
Ehrlich's diazo reaction.	368
Russo's reaction.	370
Dimethyl-amino-benzaldehyd reaction.	371
IV. MICROSCOPIC EXAMINATION.	371
Unorganized sediments.	374
Those appearing in acid urine.	374
Uric acid.	374
Sodium acid urate.	375
Potassium acid urate.	375
Xanthin.	376
Calcium oxalate.	376
Cystin.	377
Cystinuria.	377
Leucin.	378
Tyrosin.	379
Calcium sulphate.	380
Bilirubin.	380
Hippuric acid.	380
Neutral calcium phosphate.	381
Fat.	381
Chyluria.	381
Those appearing in alkaline urine.	382
Ammonium urate.	382
Calcium tri-phosphate.	382
Magnesium phosphate.	383
Magnesium-ammonium phosphate.	383
Calcium carbonate.	384
Organized sediments.	384
Mucoid material.	384
Epithelial cells.	384
Pus-cells.	386
Pyuria.	386
Vitali's test.	388
Donné's test.	389

	PAGE
Enumeration of pus-cells.	389
Red blood-cells.	389
Hematuria.	389
Casts	391
True casts.	391
Hyaline casts.	391
Granular casts	392
Waxy casts.	393
Fibrinous casts.	394
Epithelial casts.	394
Fatty casts.	394
Blood-casts.	394
Pus-casts	394
Cylindroids	395
Pseudo-casts.	396
Cylindruria	396
Spermatozoa	398
Tissue fragments	398
Bacteria	398
Bacilluria	401
Parasites.	402
V. CALCULI.	402
Uric acid calculi	403
Ammonium urate calculi.	403
Calcium oxalate calculi	403
Heller's table for analysis	404
Phosphatic calculi.	405
Calcium carbonate calculi	405
Cystin calculi.	405
Xanthin calculi.	405
Urostealith calculi.	406
VI. FUNCTIONAL DIAGNOSIS.	406
Cryoscopy.	407
Electric conductivity	408
Mosenthal's Test Meal for Renal Function	408
Phloridzin test	412
Phenolsulphonephthalein test.	412
Urea concentration test	414

CHAPTER VII

Secretion of the Genital Organs

I. MALE SECRETIONS	416
General considerations.	416
Microscopic examination.	417
Pathologic variations.	418
Medico-legal aspects.	419
Florence's test	419
Barberio's test	420
Precipitin test	420
II. FEMALE SECRETIONS.	421
Vaginal secretions.	421
Microscopic examination.	422
Pathology	422

	PAGE
Blenorrhœa.	422
Purulent secretions	423
Fetid secretions.	423
Uterine secretions.	424
Menstruation.	424
The lochia.	424
Amniotic fluid	425
Abortion.	425
Vesicular mole	425
Carcinoma.	426

CHAPTER VIII

The Blood

I. GENERAL CONSIDERATIONS.	427
II. PHYSIOLOGY AND CHEMISTRY.	428
Blood formation and blood-forming organs.	428
Total volume of blood.	429
Volume relations of cells to plasma.	432
Methods of obtaining blood	434
Physical properties	435
Color	436
Odor	437
Reaction.	437
1. Van Slyke and Cullen's Method for CO ₂	441
2. Van Slyke, Stillman and Cullen's Titration Method.	445
3. Electrometric Methods for P _H	448
4. Colorimetric Methods for P _H	448
(a) Levy, Rowntree and Marriott's Method.	453
(b) Marriott's Method for Alkali Reserve	455
Specific gravity.	457
Viscosity.	459
Coagulation	460
Osmotic pressure and cryoscopy	464
Electric conductivity	466
Chemical properties	466
Total solids	468
Blood pigments.	469
Hemoglobin	469
Pseudo-hemoglobin	470
Oxy-hemoglobin	470
Met-hemoglobin	471
Method of Stadie.	471
Carbon monoxid-hemoglobin.	473
Method of Van Slyke and Salvesen	473
Carbon-dioxid-hemoglobin.	474
Sulph-hemoglobin.	475
Decomposition products.	475
Hematin.	476
Hematoporphyrin.	476
Hematoidin	477
Hemosiderin	477
Malarial pigment.	477
Estimation of hemoglobin.	477

	PAGE
Direct methods	478
Method of Van Slyke	478
Indirect methods	481
Hemometer of Fleischl-Miescher	482
Hemoglobinometer of Dare	485
Hemometer of Sahli	486
Hemoglobinometer of Oliver	487
Hemoglobinometer of Tallqvist	489
Variations in amount of hemoglobin	490
Oligo-chromemia	491
Color index	491
Proteins of the blood	492
Other nitrogenous constituents	495
Total nitrogen	495
Total non-protein nitrogen	495
Method of Folin and Wu	496
Urea	501
Method of Van Slyke and Cullen	501
of Folin and Wu	501
Uric acid	508
Method of Benedict	508
of Folin and Wu	511
Benedict's modification of above	513
Ammonia	516
Creatinin	518
Method of Folin and Wu	520
Amino-acids	521
Method of Folin	522
Carbohydrates	523
Glucose	523
Method of Folin and Wu	526
Method of Benedict	528
Glycogen	529
Fats and fatty acids	529
Cholesterol	530
Method of Bloor	532
Method of Bloor and Knudson	533
Acetone	534
Biliary constituents	534
Inorganic constituents	535
Chlorids	535
Method of Van Slyke and Donleavy	537
Method of Whitehorn	539
Phosphates	540
Methods of Bell and Doisy	541
Method of Tisdall	542
Calcium	544
Method of Kramer and Tisdall	545
Iron	546
Blood gases	547
Ferments of the blood	548
Enumeration of the cells	548
Hemocytometer of Thoma-Zeiss	549
Hemocytometer of Durham	561
Hemocytometer of Oliver	562
III. MORPHOLOGY OF THE BLOOD	563

	PAGE
Examination of fresh blood.	564
Preparation of smears.	565
Fixation of smears.	567
Staining methods.	570
Erythrocytes.	580
Appearance and structure	580
Size and shape	582
Nucleation.	584
Number.	587
Normal variations.	587
Pathological variations	589
Oligo-cythemia.	589
Poly-cythemia	590
Staining properties.	591
Degenerations.	592
Isotonicity and resistance	594
Variations in childhood and old age	595
Functions.	596
Leucocytes	597
Appearance.	597
Types in normal blood	597
Lymphocytes	597
Large mononuclears.	598
Polymorphonuclear neutrophiles	599
Polymorphonuclear eosinophiles.	601
Polymorphonuclear basophiles	602
Types in pathological blood	603
Myelocytes.	603
Irritation forms.	604
Degeneration forms.	604
Differential counting	605
Number.	607
Leucocytosis	607
Physiological.	608
Pathological	610
Mixed leucocytosis.	613
Lymphocytosis.	614
Eosinophilia	615
Mast-cell type	616
Leucopenia.	617
Variations in infancy and childhood.	618
Functions	618
Blood-plates	619
Appearance.	619
Size	620
Number.	620
Staining properties	621
Function.	621
Hemoconien	621
Morphology of the blood-forming organs.	621
IV. PATHOLOGY OF THE BLOOD.	624
Special	624
Anemia.	624
Primary.	624

	PAGE
Simple primary anemia	624
Chlorosis.	625
Progressive pernicious anemia.	627
Splenic anemia.	629
Anemia infantum pseudo-leukemica.	630
Leukanemia	630
Aplastic anemia.	631
Secondary	631
Acute hemorrhage.	632
Chronic hemorrhage.	633
Inanition	633
Intestinal parasites	634
Fever	635
Blood poisons	635
Leukemia	636
Spleno-myelogenous type	636
Lymphatic type.	639
Acute type.	640
Pseudo-leukemia.	641
Hodgkins' disease.	641
Tuberculosis of the lymph glands.	642
Lympho-sarcoma	642
Gummatous lymphoma	643
General	643
Blood changes following surgical intervention.	643
Constitutional diseases.	644
Diabetes mellitus.	644
Gout	645
Addison's disease.	646
Rickets	646
Myxedema.	646
Acute infections	646
Pneumonia.	646
Typhoid fever	647
Scarlet fever	650
Measles	651
Variola	652
Diphtheria.	653
Pertussis.	653
Rheumatism.	654
Chronic infections.	655
Tuberculosis	655
Syphilis.	655
Leprosy.	656
Carcinoma.	656
Effects of splenectomy.	658
V. PARASITOLOGY OF THE BLOOD	658
Malaria	658
Examination of fresh blood.	660
Tertian parasite	660
Quartan parasite	663
Estivo-autumnal parasite.	664
Stained specimens.	665
Sporogony.	669

	PAGE
General hematological changes	670
Relapsing fever.	671
Sleeping sickness	672
Kala-azar	674
Filariasis.	674
Syphilis.	676
Cultivation of spirochete pallidum	679
Yellow fever	681
Infectious jaundice	683
Rocky mountain spotted fever	686
Distomiasis	687
VI. BACTERIOLOGY OF THE BLOOD	687
Technic	688
Organisms found in the blood.	689
Bacillus Typhosus.	689
Bacillus Paratyphosus.	690
Bacillus Coli Communis.	691
Pneumococcus	692
Streptococci	694
VII. SERUM PATHOLOGY	696
Ehrlich's side-chain theory	697
Phagocytosis.	701
Opsonins.	701
Allergic reactions.	703
Tuberculin reactions.	704
Luetin reactions	706
Schick reaction.	708
VIII. SERO-DIAGNOSIS	710
Agglutination reactions	710
Gruber-Widal test.	711
Method of Bass and Watkins	715
Diseases other than typhoid	717
Precipitin reaction	718
Complement-fixation test.	721
Wassermann reaction	724
Noguchi method	734
Kolmer's standardized method	739
Diseases other than syphilis	755
Abderhalden's sero-diagnosis.	757
Of pregnancy.	757
Of other conditions	769
Herman-Perutz reaction.	771
Coagulo Reaction.	773
Tests before transfusion	777
Grouping of Blood	779
IX. MEDICO-LEGAL ASPECTS.	783
Red cells.	783
Guaiac test.	784
Schaer's test	784
Phenolphthalin test.	785
Teichmann's test.	787
Spectroscopic examination.	787
Precipitin test	788
X. VALUE AND LIMITATIONS OF BLOOD EXAMINATIONS	788

CHAPTER IX

Transudates and Exudates

	PAGE
I. GENERAL CONSIDERATIONS.	790
II. PHYSICAL AND CHEMICAL PROPERTIES	791
Serous exudates.	792
Chylous exudates.	793
Chyloid exudates.	793
Hemorrhagic exudates.	793
Purulent exudates.	794
Putrid exudates.	794
III. BACTERIOLOGY	795
Tubercle bacilli.	795
Inoscopy.	795
Gonococci	795
Smegma bacilli.	798
Ducrey's bacilli.	798
Spirochæta pallidum.	798
IV. CYTOLOGY	803
Technic	803
Cytology of normal fluids	804
Cytology of pathological fluids.	805
Pleural exudates	805
Primary tubercular pleurisy	805
Secondary tubercular pleurisy	806
Pneumococcus pleurisy	806
Streptococcus pleurisy.	806
Typhoid pleurisy	806
Malignant pleurisy	806
Nephritic and cardiac pleurisy	807
Peritoneal exudates	807
V. CYST FLUIDS.	807
Ovarian cysts.	807
Serous cysts	807
Myxoid or colloid cysts	808
Papillary cysts	809
Dermoid cysts	809
Parovarian cysts	809
Hydrocele	809
Spermatocele.	809
Hydronephrosis.	809
Hydatid cysts	810
Pancreatic cysts	810
VI. CEREBROSPINAL FLUID.	810
Lumbar puncture	811
Physical Properties	812
Chemical Properties	813
Microscopic examination.	814
Epidemic cerebrospinal meningitis	815
Tubercular meningitis.	816
Acute anterior poliomyelitis	817
Cerebrospinal syphilis.	818
Noguchi's butyric acid test.	818
Noguchi's new method.	819

	PAGE
Nonne's test	820
Ross-Jones test.	820
Lange's colloidal-gold test	821
Colloidal benzoin test	823

CHAPTER X

Secretion of the Mammary Glands

I. GENERAL CONSIDERATIONS	825
II. PHYSICAL AND CHEMICAL PROPERTIES	826
Appearance and color	827
Specific gravity.	827
Reaction.	828
Coagulation	828
Total solids	828
Ash.	828
Protein	829
Total protein.	829
Method of Sebelien	829
Method of Boggs.	829
Casein.	830
Albumin and globulin.	830
Fat.	831
Babcock's method	831
Extraction method	832
Lactose	832
Preservatives in cow's milk.	833
Sodium carbonate.	833
Salicylic acid.	834
Formaldehyde	834
Boric acid and borax	834
III. BACTERIOLOGICAL EXAMINATION OF MILK	834

CHAPTER XI

Clinical Bacteriology

I. GENERAL CONSIDERATIONS.	837
II. STERILIZATION	838
III. PREPARATION OF CULTURE MEDIA	839
IV. INCUBATION	843
V. PREPARATION OF CULTURES	844
VI. STAINING	846
VII. IDENTIFICATION OF ORGANISMS	848
Diphtheria Bacillus.	848
Influenza Bacillus.	850
Pertussis Bacillus.	851
Typhoid Bacillus	852
Colon Bacillus	852
Dysentery Bacillus	855
Pathogenic Anerobes	857
(a) Bacillus Aërogenes Capsulatus	858
(b) Bacillus Oedematis Maligni	860
(c) Bacillus Oedematiens.	861
(d) Bacillus Histolyticus	861

	PAGE
(e) <i>Bacillus Fallax</i>	861
(f) <i>Bacillus Sporogenes</i>	862
(g) <i>Bacillus Tetani</i>	863
(h) <i>Bacillus Botulinus</i>	863
<i>Cholera Spirillum</i>	864
<i>Pneumococcus</i>	865
<i>Streptococcus</i>	866
<i>Staphylococcus</i>	869
<i>Gonococcus</i>	869
<i>Meningococcus</i>	871
<i>Micrococcus Catarrhalis</i>	873
VIII. VACCINES	873
Preparation of vaccines	874
Stock vaccines	874
Mixed vaccines.	876
Autogenous vaccines	876
Anti-typhoid vaccination	877
INDEX.	879

LIST OF ILLUSTRATIONS

PLATES

	TO PAGE	PAGE
I. Tubercle Bacilli in Sputum.	20	20
II. Streptococcus Pyogenes.	28	28
III. Leptothrix and Spirocheta Buccalis (Unstained)	38	38
IV. Diphtheria Bacilli Showing Polar Staining.	42	42
V. Endamebæ Gingivalis (Gros.)	46	46
VI. Koch-Weeks Bacillus.	52	52
Morax-Axenfeld Diplobacillus	52	52
VII. Trachoma Bodies of Prowazek-Greeff.	53	53
VIII. Vegetable Cells found in Feces.	168	168
IX. Osazons.	326	326
X. Ammonium Urate Crystals	382	382
XI. Waxy Casts.	394	394
XII. Mucous Threads in Urine (Unstained)	396	396
XIII. Cystitis Due to Colon Bacillus.	401	401
XIV. Staphylococcus Cystitis.	402	402
XV. Absorption Spectra.	470	470
XVI. Absorption Spectra.	471	471
XVII. Fresh Normal Blood	563	563
XVIII. Types of Red Cells.	584	584
XIX. Ring Bodies in Red Cells	594	594
XX. The Leucocytes	598	598
XXI. Iodophilia.	605	605
XXII. Polynuclear Leucocytosis	608	608
XXIII. Chlorotic Anemia	626	626
XXIV. Blood in Pernicious Anemia.	628	628
XXV. Blood in Leukanemia.	630	630
XXVI. Blood in Spleno-myelogenous Leukemia.	637	637
XXVII. Lymphatic Leukemia.	640	640
XXVIII. The Tertian Parasite (Unstained)	661	661
XXIX. The Quartan Parasite (Unstained)	663	663
XXX. The Estivo-autumnal Parasite (Unstained)	664	664
XXXI. Tertian Parasite (Stained).	666	666
XXXII. Estivo-autumnal Parasite (Stained).	667	667
XXXIII. Gonococci in Urethral Discharge.	797	797
XXXIV. Spirocheta Pallidum in Tissue.	801	801
XXXV. Exudate from Tubercular Pleurisy	807	807
XXXVI. Exudate in Pneumonic Pleurisy	807	807
XXXVII. Exudate in Malignant Pleurisy	808	808

FIGURES

	TO FACE PAGE
1. Curschmann's Spirals	7
2. Objects Found in the Sputum	10
3. Aspergillus Fumigatus.	15
4. Micrococcus Catarrhalis.	15
5. Budding Forms of Blastomycetes.	16
6. Diplococcus Pneumoniæ.	26
7. Friedländer's Bacillus	28
8. Bacillus Influenzæ.	29
9. Actinomyces.	30
10. Paragonimus Westermanii	31
11. Ovum of Paragonimus Westermanii.	33
12. Vincent's Spirillum and Bacillus	44
13. Oidium Albicans	45
14. Stomach Tube	56
15. Turcks' Aspiration Apparatus.	57
16. Boas-Oppler Bacillus	69
17. Strauss' Separatory Funnel	83
18. Sahli's Desmoid Bag	95
19. Normal Feces	106
20. Boas' Stool-Sieve.	112
21. Schmidt's Fermentation Apparatus	133
22. Cholera Spirilla.	137
23. Bacillus Typhosus.	139
24. Amœba Coli	145
25. Coccidium Hominis.	146
26. Trichomonas Intestinalis.	149
27. Cercomonas Hominis	150
28. Megastoma Entericum.	150
29. Balantidium Coli.	152
30. Tænia Solium	152
31. Tænia Saginata.	154
32. Tænia Cucumerina	154
33. Tænia Nana	154
34. Tænia Diminuta	155
35. Ovum of Tænia Diminuta	155
36. Tænia Echinococcus.	156
37. Hydatid Cyst	156
38. Bothriocephalus Latus.	157
39. Dibothriocephalus Cordatus	157
40. Ascaris Lumbricoides	158
41. Ascaris Mystax	159
42. Oxyuris Vermicularis	160
43. Strongyloides Intestinalis	161

	TO FACE PAGE
44. <i>Trichiuris Trichura</i>	162
45. <i>Trichinella Spiralis</i>	163
46. Tail of <i>Uncinaria Duodenalis</i>	164
47. Anterior End of <i>Uncinaria Duodenalis</i>	164
48. Tail of <i>Uncinaria Americana</i>	166
49. Anterior End of <i>Uncinaria Americana</i>	166
50. Parasitic Bodies, Ova, and Larvæ.	167
51. <i>Fasciola Hepatica</i>	170
52. <i>Fasciolopsis Buski</i>	171
53. <i>Opisthorchis Felineus</i>	172
54. <i>Opisthorchis Sinensis</i>	173
55. <i>Eustrongylus Gigas</i>	174
56. <i>Acarus Scabiei</i>	175
57. <i>Demodex Folliculorum</i>	176
58. <i>Leptus Autumnalis</i>	176
59. <i>Pediculus Capitis</i>	177
60. <i>Pediculus Vestimenti</i>	177
61. <i>Pediculus Pubis</i>	177
62. <i>Pulex Irritans</i>	178
63. <i>Pulex Penetrans</i>	179
64. <i>Achorion Schönleini</i>	180
65. Normal Hair.	181
66. <i>Trichophyton Endo-ectothrix</i>	181
67. <i>Microsporon Audouini</i>	182
68. Mycelial Threads of <i>Blastomycetes</i>	183
69. Budding Forms of <i>Sporothrix Schenckii</i>	184
70. Urinometer and Cylinder.	203
71. Volumetric Flasks.	219
72. Kjeldahl's Nitrogen Apparatus.	236
73. Doremus' Ureometer	244
74. Doremus-Hinds Ureometer.	244
75. Folin's Urea Apparatus	245
76. Schlösing's Ammonia Apparatus	252
77. Folin's Ammonia Apparatus	253
78. Folin's Absorption Bulb.	254
79. Ruhemann's Uricometer.	264
80. Duboscq Colorimeter	270
81. Conical Test-glass	298
82. Horismascope	298
83. Esbach's Albuminometer	302
84. Laurent Polariscopes.	333
85. Diagrammatic Representation of the Course of Light through the Laurent Polariscopes.	334
86. Einhorn's Saccharometer.	335
87. Lohnstein's Fermentation Tube for Undiluted Urine	337
88. Lohnstein's Fermentation Tube for Diluted Urine	337

89. Purdy Electric Centrifuge	371
90. Sediment Tube.	372
91. Percentage Centrifuge Tube	372
92. Various Forms of Uric Acid	374
93. Acid Sodium Urate	375
94. Xanthin.	375
95. Calcium Oxalate	376
96. Cystin.	377
97. Pure Leucin	378
98. Impure Leucin	379
99. Tyrosin	379
100. Calcium Sulphate.	380
101. Bilirubin.	380
102. Cholesterin.	382
103. Magnesium-Ammonium Phosphate	383
104. Calcium Carbonate	384
105. Urinary Epithelium.	385
106. Pus Coruscules.	387
107. Hyaline Casts	391
108. Granular Casts.	393
109. Epithelial Casts.	394
110. Fatty Casts	395
111. Blood, Pus, Hyaline, and Epithelial Casts	396
112. Cylindroids	397
113. Scolex and Hooklets of <i>Tænia Echinococcus</i> in Urine	401
114. Ova and Miracidium of <i>Schistosomum Hematobium</i>	402
115. Normal Semen	417
116. Chorionic Villi	425
117. Daland's Hematocrit	432
118. Hematocrit Tube.	433
119. Blood Needle.	435
120. Van Slyke Carbon Dioxide Apparatus	443
121. Pycnometer	459
122. Boggs' Coagulometer	463
123. Beckmann Apparatus	464
124. Direct-vision Spectroscope.	470
125. Hemometer of Fleischl-Miescher	482
126. Hemoglobinometer of Dare.	485
127. Hemoglobinometer of Dare with electric attachment	485
128. Method of Filling the Dare Blood Pipet	486
129. Hemometer of Sahli.	487
130. Hemoglobinometer of Oliver	488
131. Tallqvist's Hemoglobinometer	489
132. Thoma-Zeiss Counting Chamber	549
133. Diluting Pipets.	550
134. Ruled Surface of Thoma-Zeiss Counting Chamber.	551

135. Türk's Ruling of Counting Chamber	552
136. Plan of Counting the Cells.	558
137. Cross-section of Durham's Blood Pipet	561
138. Oliver's Hemocytometer.	562
139. Preparation of Blood-smears with Glass Slides	565
140. Preparation of Blood-smears with Cigarette Paper	566
141. Ehrlich Forceps.	566
142. Pinch Forceps	567
143. Oven for Fixing Blood-films	568
144. Normal Blood Showing Rouleaux Formation and Fibrin Network	580
145. Cycles of the Malarial Parasite.	668
146. Spirillum of Obermeier.	672
147. Trypanosoma Gambiense	673
148. Filaria Bancrofti	675
149. Spironema Pallidum and Refringens	678
150. Ultra-condenser of Reichert.	679
151. Schistosomum Hematobium	685
152. Illustrating the Mechanism of Toxin-cell Union.	697
153. Illustrating the Elaboration and Action of Antitoxin.	698
154. Illustrating the Mechanism of Hemolysis.	699
155. Illustrating the Mechanism of Antihemolysis.	700
156. Bacillus Typhosus at Beginning of Widal Test	710
157. A Pseudo-Widal Reaction	711
158. A Positive Widal Reaction.	711
159. Hemin Crystals from Human Blood.	785
160. Lumbar Puncture.	812
161. Diplococcus Intracellularis Meningitidis	815
162. Normal Milk and Colostrum.	825
163. Babcock Milk and Cream Bottles.	831
164. Bottle for Human Milk.	831
165. Soxhlet Apparatus	833
166. Wolfhügel's Colony Counter	835
167. Lautenschlager Hot Air Sterilizer.	837
168. Autoclave.	838
169. Arnold Sterilizer	839
170. Incubator	844
171. Platinum Needles.	845
172. Apparatus for Anærobic Cultivation.	846

DIAGNOSTIC METHODS

CHAPTER I

THE SPUTUM

I. GENERAL CONSIDERATIONS

At the present time the examination of the sputum is more or less limited to the search for various specific organisms, especially the bacilli of tuberculosis and of pneumonia. This is very much to be regretted, as frequently the appearance, amount, consistency, color, and other characteristics are of great aid and have led our older brothers to correct diagnoses before the days of microscopic examination.¹

The sputum, strictly speaking, should be considered as the material which comes from the respiratory passage anywhere along its course. It may be, therefore, of laryngeal, bronchial, or alveolar origin. More commonly, however, we find the sputum considered by the general practitioner and by the laity as anything which is expectorated, so that specimens which consist of nothing more than salivary secretion are frequently sent to laboratories for examination.² In some cases, especially of inflammation of the naso-pharynx or perforation from neighboring organs, this buccal secretion may be mixed with material from the nose, mouth, ear, or esophagus. For these reasons, if for no others, it is absolutely essential that frequent examinations of sputum, in the strict sense, be made before a negative diagnosis of a suspected condition may be given.

As a rule, any sputum at all should be considered pathological, as normal persons raise little or nothing from the lungs at any time. By this is not meant that serious disease may obtain when a small amount of sputum exists, as patients suffering with catarrhal conditions of the naso-pharynx frequently have an accumulation of material, which has settled in the bronchial tubes over night and is raised in the morning. Those of us who are unfortunate enough to live in atmospheres which are loaded with soot and dirt frequently raise a certain amount of sputum which arises from an increased activity of the mucous membrane of the respiratory passages to compensate for the dryness and irritation which these foreign substances have caused. This morning sputum is small in amount and is in the form of large, tough, elastic masses which very much resemble boiled sago. These masses are at times extremely dark in color, due to the dirt which has been taken in with the inhaled air. In such sputum we find much mucus, degenerated epithelium, pus cells and various micro-organisms. These organisms are

¹ See Laird, *Jour. Am. Med. Assn.*, 1915, LXIV, 427; Warnecke, *Deutsch. Med. Wchnschr.*, 1920, XLVI, 1439.

² See Wooley, *Jour. Lab. and Clin. Med.*, 1922, VII, 308.

rarely of pathologic significance, although many pathogenic types may be present.

The sputum should be collected in receptacles which may be completely and easily disinfected. The best receivers for the sputum are the ordinary paper spit-cups which can be burned as occasion may demand. The practice of expectorating upon cloths is only to be advised when these cloths are immediately burned. In the spit-cup should always be contained a certain amount of disinfectant, such as dilute carbolic acid or corrosive sublimate solution, so that no chance of transference of infection may arise. Neglect of such precautions has frequently led to serious consequences in the case of the healthy members of the family. When sputum is to be collected for examination, the material raised by coughing should be received in a wide-mouthed bottle which contains no disinfectants, as these agents coagulate the protein material of the infecting organism and in some cases change its staining characteristics. Immediately after examination of such material it should, of course, be treated with the disinfecting solution.

II. PHYSICAL AND CHEMICAL CHARACTERISTICS

Amount.

Some general idea of the amount of sputum expectorated is always advisable. It is rarely necessary, however, to make any collection of the material during the 24 hours' period, as one can usually gain the information by questioning the patient or nurse as to the amount passed from time to time. The amount of sputum expectorated in 24 hours depends very much upon the nature of the pathologic condition. In cases of so-called dry bronchitis, diffuse bronchitis, early tuberculosis, and occasionally of lobar pneumonia, the sputum is so viscid that there is practically none obtained. In cases of chronic bronchitis, tuberculosis with cavity formation, and bronchiectasis we find large amounts; while in cases of lung abscess or of perforating pleurisy, blood or pus may flow from the mouth in very large quantities. The absolute amount of sputum may, therefore, vary from a cubic centimeter to a liter or more. An extensive expectoration will naturally have more or less serious effect upon the patient's general nutrition, so that we are not surprised to find cases in which as high as 5 per cent. of the total nitrogen eliminated passes from the system in the sputum (Lenz).

Consistency.

As a rule, the consistency of the sputum varies from that of a liquid to a highly tenacious material, inversely as the amount of sputum. This latter statement holds in most cases with the exception of pneumonia, in which we have an extremely tenacious sputum and, also, a very abundant one. Just what substance induces this extreme tenacity is uncertain, but it may be generally said that mucin is the causative factor, although the tenacious pneumonic sputum shows very little mucin. According to Kossel, the tenacity may be due to the presence of nuclein derivatives. In the early stages of acute bronchitis, or bronchial asthma, and in whooping-cough the sputum is usually very

tenacious and ropy; while in edema of the lungs, pulmonary abscess, putrid bronchitis, and pulmonary gangrene it is very watery and contains large numbers of pus-cells.

Reaction.

The fresh specimen of sputum is usually alkaline in reaction. However, in cases in which the sputum has remained in the lungs for some time, as in cavity formation, the reaction is acid.

Color.

The color of the sputum may range from that of a colorless material to one showing any of the tints of the rainbow. These colors are due to admixtures of various abnormal products with the sputum. A bright red sputum is significant of the presence of blood, according to Traube, unchanged red blood-cells necessarily being present. The amount of blood may vary from a light streaking of the sputum to one showing a deep red, rusty, or prune-juice color. Such bloody sputa are found after trauma, pneumonia, gangrene, hemorrhagic infarction of the lungs, chronic passive congestion, as well as adventitious mixture of sputum with nasal or pharyngeal material containing blood. The blood may be due to rupture of a vessel and may then constitute the condition known as pulmonary hemorrhage. This condition of hemoptysis¹ is frequently confounded with that of hematemesis and is differentiated by the fact that the blood in hemoptysis is frothy, bright red in color, alkaline in reaction, and usually associated with mucopurulent material; while the blood in hematemesis is frequently dark and grumous, usually clotted and acid in reaction. In sputa which are tinted by changed hemoglobin, the color is most varied. Many oxidation products of hemoglobin are found in the sputum, depending upon the time which the sputum has lain in the lungs. Thus, for instance, in pneumonia we find a rusty, prune-juice colored sputum, whose color seems to be due to an unknown derivative of hemoglobin. The shade of color in pneumonia may, however, range from red through brown to green. In cases of mitral disease associated with passive congestion of the lungs we find, frequently, a light brown color, due to the presence of hematin granules in the epithelial cells.

In many cases of abscess of the liver which perforates into the lung or in catarrhal jaundice, bile pigments may be found in the sputum which may give rise to various colored sputa from red through blue to green. Although, as shown in a later table, the bile pigments and hemoglobin are very closely related chemically, yet the clinical significance of the appearance of bile pigment in such cases as the above is very important. In many cases of true jaundice the sputum, in case any exists, may show a distinct grass-green color due to the presence of oxidized bile pigments. This same green color frequently appears in the sputum, however, in cases of croupous pneumonia during lysis. In this latter case the color is due, probably, to the same pigment which is oxidized before expectoration. In these cases of pneumonia with green sputum and no jaundice, a fresh involvement of the lung is usually

¹ See van der Hoeven-Léonhard, *Arch. internat. de laryngol.*, 1913, XXXV, 831.

associated with a rusty sputum. Besides jaundice and pneumonia we may have, as causes of green sputum, certain chloromata of the lungs as well as the development of chromogenic bacteria within the lungs. This action of chromogenic bacteria is not always observed when the sputum is expectorated, but may appear only after the sputum has stood. The presence of the bacillus pyocyanus may give a sputum which is brilliant blue or greenish in tint.

Sputa very frequently show changes in color as well as consistency due to various substances inhaled. Thus we find a distinctly black sputum, in cases of anthracosis, in coal-miners and in many city residents. So frequent is this occurrence that the lung tissue may even be invaded by the coal pigment.¹ Workers in bronze and brass as well as other metals frequently show a sputum tinged red with ferric oxide, arising from a condition of the lung known as siderosis.² Stone-cutters frequently show much stone-dust in the sputum which is characteristic of the condition of chalicosis, stone-cutters' phthisis, or grinders' rot. Workers in flour mills and in bakeries frequently expectorate doughy masses, while those in cotton mills show the presence of cotton fibers in their sputum. Finally it may be said that the color of the sputum may be changed by the presence of certain foods, such as milk, eggs, and chocolate, while tobacco users frequently have a sputum tinged dark brown.

Odor.

Ordinarily the sputum has no odor unless it has stagnated either in the receiving cup or in the system. Such old sputum sometimes has a very distinct putrefactive odor. The odor of sputum in tuberculosis and bronchiectasis is peculiarly heavy and sweet, while that in putrid bronchitis is often extremely offensive. In cases of perforating empyema a peculiar cheese-like odor is observed. While these odors are not characteristic in themselves, they are usually more or less significant of the condition with which they are commonly associated.

Character of the Sputum.

The character of the sputum has reference more to the apparent composition of the sputum than to its consistency. Air is usually present in the sputum in various amounts, so that one may judge, from the size of the air-bubbles, of the size of the bronchi from which the sputum came. The sputum from cavities and large bronchi contains no air and, therefore, sinks in water. This is the so-called *sputum fundum petens*.

Sputum known as mucoid sputum is glairy, transparent, and tenacious, becoming cloudy on the addition of acetic acid due to the precipitation of mucin. This type of sputum is found particularly in acute bronchitis and in asthma.

A mucopurulent sputum is one containing both pus and mucoid material. Small amounts of pus give a whitish color, either to the whole or to portions of the sputum, the pus being observed in masses or in streaks through the mucoid material. Larger quantities of pus give a yellowish or occasionally a

¹ See Filadoro, *Med. nuova*, 1913, IV, 133; also, Haythorn, *Jour. Med. Research*, 1913, XXIX, 259; Klotz, *Am. Jour. Pub. Health*, 1914, IV, 887. Jackson (*Penn. Med. Jour.*, 1922, XXV, 613) calls attention to the gray "moss agate" appearance of the sputum in chronic tracheitis.

² See Gigon, *Beitr. z. path. anat. u. z. allg. Path.*, 1912, LV, 46.

yellowish green tinge to the sputum. In this latter type the pus and mucus seem to be mixed homogeneously. In the sputum from cavities we find the mucopurulent material arranging itself flatly like a coin, constituting the so-called "*nummular*" sputum.

Purulent sputum is found in cases of ruptured empyema, abscess of the lung, and in some cases of bronchiectasis. This purulent sputum differs from the mucopurulent type in the fact that the pus is much more abundant and is almost in the pure state, being mixed with a small amount of tenacious mucus.

In some cases, especially in edema of the lungs, a sputum is obtained, known as serous sputum, which is colorless and quite frothy. This sputum resembles very closely the ordinary salivary secretions and should not be confused with it.

In many cases, especially in putrid bronchitis, gangrene of the lung, and bronchiectasis, the sputum on being voided into a cylinder will separate into three distinct layers, occasionally into four. The upper layer is of frothy mucus; a second layer, which is not always present, consists of certain albuminous material which hangs in long shreds down into the third layer, which consists of the sero-pus and is usually opaque and watery. The bottom layer contains the morphological elements, pus, tissue shreds, and bacteria.

Besides the varieties of sputum named above, the admixture of blood may give rise to sputum which is known as sanguinous sputum or as sanguino-mucopurulent or purulent sputum.

Chemical Properties.

The chief chemical examination is applied to the detection and estimation of the amount of albumin and of mucin in the sputum. The method is as follows:¹ Treat the sputum with an equal quantity of 3 per cent. acetic acid, in order to precipitate the mucin. Shake thoroughly for a few minutes and allow to stand for 15 minutes. Filter through filter-paper until a perfectly clear filtrate is obtained, adding a known amount of water to facilitate filtration if necessary. Apply to this filtrate any of the tests for albumin given in the section on urine. If the quantitative determination is desired, use Tsuchiya's reagent as the precipitant, multiplying the result by the dilution of the sputum to obtain the true percentage of albumin.

A large amount of albumin indicates, of course, such conditions as pneumonia, pulmonary edema or perforating empyema. While the early workers regarded anything more than a slight opalescence as pathological, a trace of albumin indicating a pulmonary tuberculosis in their opinion, later investigators showed that albumin is present in the sputum whenever there is active exudation into the alveoli as well as into and under the pleura. Many more recent studies have proven that albumin may not be present in the sputum of true pulmonary tuberculosis, but is frequently seen in benign affections and when present is due, in the majority of cases, to occult blood.² For these reasons it is more than probable that such chemical tests are to be given

¹ Lesieur and Prires, Paris méd., 1911, IV, 29.

² See Wanner, Deutsch. Arch. f. klin. Med., 1903, LXXV, 347; Roger and Levy-Valensi, Presse méd., 1910, XVIII, 289; Ibid., 1911, XIX, 409; Geeraerd, Jour. de méd., de chir. et

merely a presumptive interpretation, although the presence of albumin may differentiate an active from a latent process.

Besides these albuminous principles, the sputum, especially in cases of gangrene and putrid bronchitis, contains a ferment very much resembling in its action the trypsin of the pancreatic juice (Stolnikow).¹ This ferment seems to indicate a highly destructive process in the lung tissue. Other chemical substances, such as glycogen² and fatty acids,³ are frequently found in the sputum but may be passed with mere mention. The so-called myelin granules or globules, which appear in the alveolar cells of the sputum, consist largely of fatty principles, such as protagon, lecithin and cholesterolin.

III. MACROSCOPIC EXAMINATION

While much that is included in the previous section would come properly under the head of the macroscopic examination of the sputum, the writer has reference more, in this connection, to the appearance of macroscopic elements as distinguished from those which are purely microscopic.

(1) Cheesy Masses.

Frequently one finds in the sputum small cheese-like particles which vary in size from that of a pin-point to that of a pea, the large majority being about the limit of ordinary vision. These cheesy masses are fragments of necrotic tissue and appear in the larger form in cases of abscess or gangrene of the lung, while in tuberculosis they are always small unless the cavity, from which the material is derived, is markedly necrotic. The color of these masses varies from a yellow to a black. Those fragments which come from an abscess are of yellow color due to the presence of much pus, the darker ones contain decomposition products of hemoglobin, while many of them are tinged a deep black with coal pigment. If the sputum be squeezed between two glass plates these cheesy particles or fragments can sometimes be more distinctly seen.

de pharmacol., 1910, XV, 505; Raymond, Presse méd., 1911, XIX, 675; Goodman, Arch., Int. Med., 1911, VIII, 163; Fishberg and Felberbaum, Med. Record, 1911, LXXX, 870; Fishberg, Arch. Diagnosis, 1912, V, 220; Works, Jour. Am. Med. Assn., 1912, LIX, 1537; Scott, Ibid., 1913, LX, 440; Acs-Nagy, Wien. klin. Wchnschr., 1912, XXV, 1904; Kauffmann, Beitr. z. Klin. d. Tuberk., 1913, XXVI, 269; Schmitz, Med. Klin., 1913, IX, 1163; Berkovits and Rudas, Berl. klin. Wchnschr., 1913, L, 1752; Ridge and Treadgold, Lancet, 1913, II, 382; Schneider, Zentralbl. f. inn. Med., 1913, XXXIV, 1025; Gelderblom, Deutsch. med. Wchnschr., 1913, XXXIX, 1087; Maliwa, Deutsch. Arch. f. klin. Med., 1913, CXII, 231; Hohn and Himmelberger, Jour. Am. Med. Assn., 1914, LXII, 20; Lewis, New York Med. Jour., 1914, XCIX, 1031; Melikjanz, Med. Obozr., 1914, LXXX, 819; Beitr. z. Klin. d. Tuberk., 1914, XXX, 81; Wien. klin. Wchnschr., 1914, XXVII, 653; Cocke, Am. Jour. Med. Sc., 1914, CXLVIII, 724; Hafemann, Deutsch. med. Wchnschr., 1914, XL, 1715; Benzler, Beitr. z. Klin. d. Tuberk., 1914, XXXII, 363; Glover, Brit. Jour. Tuberc., 1914, VIII, 217; Holm and Chambers, Jour. Michigan Med. Soc., 1914, XIII, 226; Callender, Med. Record, 1914, LXXXVI, 783; Fantoni, Gazz. d. osp., 1914, XXXV, 417; Löwenbein, Ztschr. f. Tuberk., 1915, XXIII, 122; Szaboky, Ibid., 352; Sniijders, Nederl. Tijdschr. v. Geneesk., 1915, I, 349; Lockwood, Jour. Am. Med. Assn., 1915, LXIV, 574. Holm and Chambers, Jour. Lab. and Clin. Med., 1916, I, 519; Salomon, Presse Méd., 1919, XXVII, 523; Médecine, 1920, I, 508; Felsani, Rif. Med., 1920, XXXVI, 1051; Burdick and Gauss, Amer. Rev. Tuberc., 1921, IV, 889; Alport, South African Med. Record, 1921, XIX, 127; Takeuchi, New York Med. Jour., 1921, CXIII, 574; Aris, Médecine, 1921, III, 233.

¹ See Eiselt, Ztschr. f. klin. Med. 1912, LXXXV, 71; also, Maliwa, Deutsch. Arch. f. klin. Med., 1914, CXV, 407.

² See Felsani, Rif. Med., 1921, XXXVII, 249.

³ See Barbaro, Gazz. d. osp., 1915, XXXV, 985. Cox, Journal-Lancet, 1916, XXXVI, 515. Felsani (Rif. Med., 1920, XXXVI, 858) reports the finding of uric acid in sputum.

They are present in largest numbers in the so-called "nummular" sputum from a tuberculous cavity.

(2) **Dittrich's Plugs.**

These masses are similar to the small caseous particles above mentioned and are frequently expectorated by perfectly normal individuals. The true plugs are distinct casts of the bronchi or bronchioles and vary in size from pin-point to that of a bean, the majority being about the size of a mustard seed. The smaller ones are opaque and yellowish-white in color, while the larger ones have a distinct gray tinge. They are usually expectorated free from pus or mucus, so that they frequently give rise to anxiety, especially in those of a hypochondriac tendency. These plugs have a distinctly disagreeable odor, which is more evident if they are crushed on the glass plates. Microscopic examination of these masses shows large clumps of bacteria, fatty acid crystals, free fat globules, and cellular detritus. Occasionally a few leucocytes are found, but these are rare, while pigment granules, either of hematogenous or extraneous origin, are sometimes observed. While such plugs are especially numerous in cases of putrid bronchitis and bronchiectasis, they are frequently found in the crypts of the normal tonsil as well as in cases of follicular tonsillitis or of ozena.

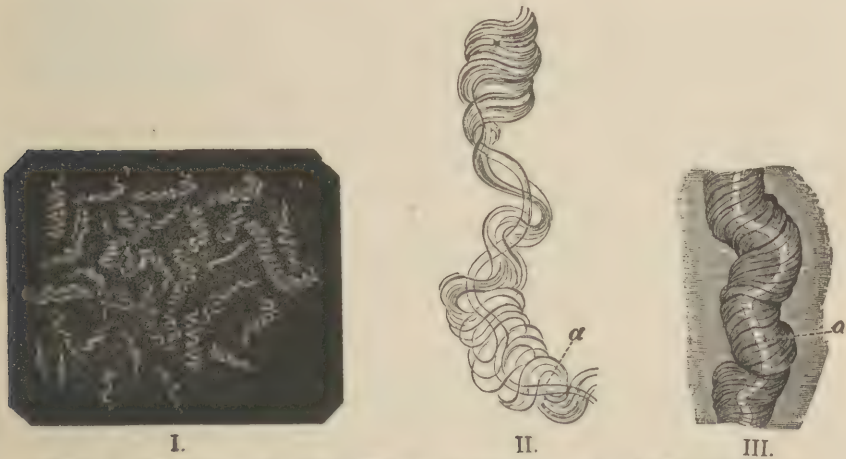


FIG. 1.—Curschmann's spirals (*Tyson after Curschmann*). I, Natural size; II and III, enlarged; a, a, central thread.

(3) **Curschmann's Spirals.**

These structures are found in the sputum in practically every case of true bronchial asthma and have been reported in acute bronchitis, croupous pneumonia, chronic pulmonary tuberculosis, and occasionally in chronic bronchitis. They are not present in every paroxysm of asthma, but are more frequently found just at the end of the paroxysm and are absent when the sputum becomes mucopurulent. They seem to be derived through true exudation from the bronchioles, as Curschmann says, a bronchiolitis exudativa. These structures are recognizable, to a certain extent, by the naked eye, but for their absolute identification a microscopic examination is essential. They

are composed of a spirally-twisted network of very delicate fibrils, in the meshes of which are numerous epithelial cells and eosinophile leucocytes. Along with these cellular bodies one frequently finds large numbers of diamond-shaped crystals, known as the Charcot-Leyden crystals, which will be discussed later. This spirally-twisted mass (the mantle) seems to be wound around a central light thread. While this is the structure of the complete spiral, we frequently find variations pointing apparently to two distinct forms. The first is the spirally-twisted strand of mucus with the enclosures above mentioned. The second is the tight spiral mass of mucus wound around a central fibre. This central fibre is very refractive and is homogeneous in structure, varying in size from $\frac{1}{2}$ to 18 microns in diameter. The length of these spirals is from 1 to 2 cm. and their breadth about 1 mm.

(4) **Fibrinous Casts.**

By the term fibrinous cast we refer more directly to true bronchial casts, which are composed of fibrin. These are observed in pneumonia, in which case they are derived from the smaller bronchioles and are brownish or reddish in color and contain many red and white blood-cells. These smaller casts vary from $\frac{1}{2}$ to 3 cm. in length. In the chronic fibrinous bronchitis we find the so-called arborescent casts which are usually whitish in color and contain many epithelial cells. These casts vary in size from 1 to 15 cm. in length by several mm. in thickness. We may have an acute form of fibrinous bronchitis accompanying various febrile conditions, so that similar casts may appear in almost any of the infectious diseases. These larger casts are fairly firm, usually have a lumen, and branch dichotomously five to ten times. Microscopic examination shows them to consist of large numbers of longitudinal fibers containing blood and epithelial cells in their meshes. They may be stained with the Weigert fibrin stain in a very beautiful way. Such staining methods show that not all the material in such casts is fibrin, so that it may be necessary to rename these structures as simple bronchial casts rather than as fibrinous forms.

Besides these fibrinous casts, one occasionally may find distinct bronchial casts composed of the mycelium of various fungi. Thus mycelial threads of the aspergillus have been reported by Osler, Devillers and Renon.

(5) **Concretions.**

This name is applied to anything, expectorated with the sputum, which has the appearance or consistency of a stone. These concretions are formed in dilated portions of the bronchi or in cavities by the calcification of the stagnated contents. Although concretions consisting of cartilaginous or osseous material are frequently found postmortem, yet there seems to be little mention in the literature of any such formations being expectorated.¹

(a) **Bronchioliths.**

These concretions are formed by the deposition of calcium salts in the stagnated contents of the bronchus or of a cavity. They may be derived from the smaller or larger bronchi, but rarely form arborescent shapes, being usu-

¹ See Cade and Guérin, *Lyon méd.*, 1914, XLVI, 221.

ally irregular and varying in size from a pin-head to that of a walnut. They are usually single, but may be multiple. They vary in consistency from that of chalk to a stone, and may be expectorated in small numbers over long periods of time.¹

(b) **Pneumoliths.**

These lung stones are in the majority of cases of tuberculous origin. They usually arise by the calcification of caseous areas which later ulcerate into a bronchus and are expectorated unless too large. They may, also, arise by the calcification of a pulmonary cavity or of a bronchial lymph-gland. These lung stones consist of the carbonates, phosphates, and sulphates of calcium and magnesium, in some one or more salts predominating, while in others still different combinations may exist. These pneumoliths either have a chalky or a calcareous consistency and vary in size from that of a pin-head to that of a tennis-ball. These lung stones are usually expectorated *en masse* or in the form of smaller portions of a larger stone. In some cases these smaller stones may reach the number of 500 (Portal).

(6) **Echinococcus Membranes.**

Rarely one may find in the sputum fragments of the walls of echinococcus cysts or their contents. These may come from a perforating cyst of the liver, kidney, or lung. The presence of the laminated membrane and of the parasitic scolices and hooks makes it possible to arrive at an absolute diagnosis of the origin of such material. The membrane is thick, tough, and of a porcelain-like color and may show a laminated or fibrillated structure. The parasite is discussed in a later section.²

(7) **Foreign Bodies.**

Examination of the sputum may reveal the presence of material which has lodged in the air-passages and been retained for long periods of time. Such bodies are coins, fish-bones, and cherry-stones. Heyfelder reports a case of the expectoration of a wooden cigar-holder 11½ years after its disappearance.

IV. MICROSCOPIC EXAMINATION

The microscopic examination of the sputum is almost the only one to which it is subjected at the present day. This is to be regretted, as much may be learned from a careful macroscopic examination. However, a microscopic examination reveals evidence which points to an absolute diagnosis more frequently than does the macroscopic examination. Before making the microscopic examination, it is wise to place the sputum in a flat-bottomed dish (Petri dish) which has half of its base blackened, so that the more suspicious particles may be selected for microscopic investigation. Some experience in this work is necessary, as one is frequently called upon to recognize material which is purely extraneous and has absolutely nothing to do with the sputum. Such material practically always comes from the buccal cavity and consists of

¹ See Jackson and Spencer, New York Med. Jour., 1921, CXIII, 461; Leuw, Schweiz. med. Wchnschr., 1921, LI, 1022.

² See Creyx, Jour. de Méd. de Bordeaux, 1920, XCI, 139.

fragments of various food-stuffs, such as bread, fruit pulp, meat fibers, vegetable tissue, and portions of tobacco leaf. Naturally, such material should not mislead one, but it very frequently does. The fragments of meat tissue contain elastic tissue fibers and may lead one to state that such material is present in the sputum, thus giving expression to the possibility of a diagnosis of incipient tuberculosis.

A portion of the sputum selected for examination is taken up with a platinum loop and spread in a thin layer upon a glass slide. It is then dried by passing the slide several times through the flame, care being taken not to burn the specimen. The smear is allowed to cool and is then stained either with Löffler's methylene blue for general purposes or with special stains for the various specific organisms.

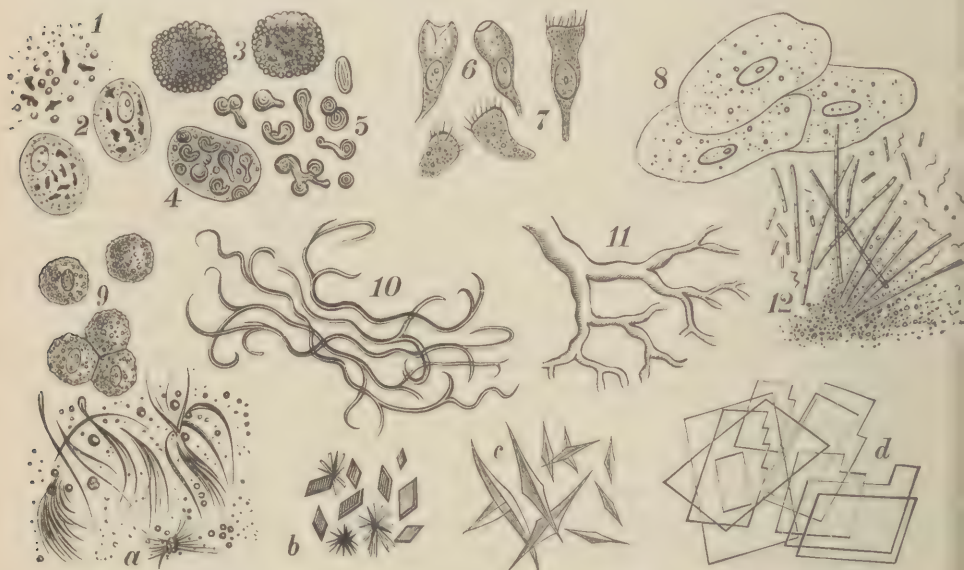


FIG. 2.—Objects found in the sputum (Landois). 1, Detritus and dust-particles; 2, pigmented alveolar epithelium; 3, fatty degenerated and partially pigmented alveolar epithelium; 4, alveolar epithelium showing myelin-degeneration; 5, free myelin forms; 6, 7, desquamated ciliated epithelium, partly changed and deprived of its cilia; 8, squamous epithelium from the mouth; 9, leucocytes; 10, elastic fibers; 11, fibrinous cast of a small bronchus; 12, leptothrix buccalis, together with cocci, bacilli, and spirochete; a, fatty acid crystals and free fatty granules; b, hematoidin; c, Charcot's crystals; d, cholesterin.

(a) Pus-cells (Leucocytes).

There is practically no specimen of sputum which does not contain leucocytes in larger or in smaller numbers. The true pus-cell is the polymorphonuclear neutrophile and appears in the sputum frequently filled with fat globules or pigment granules. In cases of asthma, the eosinophile cells¹ are very abundant, while basophile cells may occasionally obtain. Although these cells are so frequent in asthma, a diagnosis should not rest upon such evidence alone, as there seems to be a form of bronchitis, possibly of the tubercular variety, which has been named "eosinophilic bronchitis" from the

¹ See Besancon and de Jong, Bull. Acad. de Méd., 1920, LXXXIV, 253.

large number of such cells observed. In pulmonary tuberculosis, which is not associated with a mixed infection, it is very common to find the small mononuclear leucocyte (lymphocyte) in place of the polynuclear type. This finding is of such frequent occurrence that the writer is often led to search many slides for tubercle bacilli in case the organisms have not been found in the earlier specimens examined. Basophilic cells are not infrequently observed in the sputum. Pottenger reports¹ the presence of macro-phagocytes in a case of chronic hemoptysis. The cytology of the sputum shows little of diagnostic value.

The thin smears of sputum may be treated like blood-smears and stained with the same stains when one desires to study the cellular types present.

(b) Red Blood-cells.

These cells are frequently found in the sputum and may have much significance. They may occasionally arise from contamination with nasal or buccal discharges, but the true bronchial or pulmonary sputum usually shows them only in cases of hemorrhage or exudation. The rusty sputum of pneumonia contains large numbers of such cells and the hemorrhage from a tubercular cavity may be very extensive. These cells are occasionally well-preserved, but at times they are difficultly recognizable. They may be very much distorted in shape, so that their color and staining properties must be relied upon for differentiation.

(c) Epithelial Cells.

Various types of epithelial cells are found in the sputum.² Pavement epithelium may come from the mouth, pharynx, and upper larynx. Cylindrical epithelium may be derived from the nose or the bronchi. These cylindrical cells may be ciliated, but rarely does one find these ciliated forms except in cases of asthma and bronchitis. Alveolar epithelial cells are present in normal sputa as they are constantly desquamated from all the epithelial surfaces. These cells assume a large variety of forms and frequently show various types of degeneration. They are very numerous in bronchitis and in general inflammatory conditions of the lungs, but may occur in almost any condition, associated with irritation along the respiratory tract. These cells contain large numbers of granules which are probably referable to coal pigment. Such cells give the sputum a grayish or green color. An abundance of such cells in the sputum was designated in earlier times as "*phthisis melanotica*." Occasionally these alveolar cells are filled with fat globules. In other cases one finds the so-called myelin globules, which are irregular in shape, often showing concentric lines, with very little refractility, and of a dull greenish or blue appearance. The cell may be invisible and only the myelin appear as a large irregular mass. Much doubt exists as to the origin of these globules, but it is more probable that they represent simply the fatty products of degenerated protoplasm. These cells are especially frequent in the normal morning sputum, in acute or chronic influenza, and in the so-called

¹ Jour. Am. Med. Assn., 1912, LIX, 1794.

² See Otani, Jour. Exper. Med., 1917, XXV, 333.

desquamative catarrhal pneumonia, when they appear as small lumps resembling boiled sago. Occasionally free myelin globules are found in the sputum. These globules stain poorly with the aniline dyes, are stained yellow with iodine, but do not stain black with osmic acid or red with Sudan III.

Frequently these alveolar cells contain pigment granules, derivatives of hemoglobin. This material is in the form of amorphous granules of a brown color and seems to be identical with hemosiderin, but may become iron-free, when it more closely resembles hematoidin. Cells containing such granules are especially numerous in chronic heart disease and are styled, therefore, "*heart-disease cells*." They occur, however, in any condition in which red blood-cells escape into the alveoli and are found, therefore, in pneumonia, infarction of the lung, and hemorrhagic pulmonary tuberculosis.

(d) **Elastic Tissue.**

The presence of elastic tissue in the sputum is indicative of destruction of the lung tissue and in many cases is found in the sputum before one can detect tubercle bacilli. It has, therefore, some importance in the early diagnosis of tuberculosis. When this elastic tissue is grouped in masses it is usually visible to the naked eye. However, one more frequently relies upon microscopic examination for its detection. The method of Clark, as applied to the detection of elastic tissue, is usually the best one to follow. The sputum is placed upon a glass plate about 14 inches square and pressed out by a smaller one, about 6 inches square, into a thin layer. The plates are then placed upon a dark background and examined with a hand lens. Instead of these glass plates one may use Petri dishes.

The elastic tissue fibers appear either in the form of distinct strands sometimes grouped in an elongated network, in that of the alveolar type, in which the fibers preserve the outline of the alveoli and are long and branching, or in that from the arteries in which we may have a distinct sheet-like arrangement. These fibers are characterized by their undulating outline, their curling ends, their sharp edges and uniform diameter, their frequent branching, and their intense refractility. These characteristics are brought out both with the low and with the high power of the microscope. The fibrous tissue fibers are differentiated from elastic fibers by the fact that the former are present in bundles of fine wavy lines without the coarse black refractive appearance of elastic tissue. Chains of bacteria, especially the leptothrix forms, frequently interlace in such a way as to simulate the alveolar structure of elastic tissue. These chains differ, however, from elastic tissue in their refractility, in the absence of the wavy outline, and in their denser arrangement. The elastic tissue fibers derived from food substances have the same characteristic as the lung elastic fiber, with the exception that they are not arranged in the alveolar form and may at times be coarser and more irregular in outline than the pulmonary elastic tissue. Vegetable cells and fibers, as well as fatty acid crystals, which may occur in the sputum, should not mislead one into assuming the presence of elastic tissue.

Should one wish to stain elastic tissue, he may use the orcein stain of

Unna-Tänzer. This stain consists of one gram of orcein dissolved in a mixture of 80 c.c. of 95 per cent. alcohol and 35 c.c. of distilled water, 40 drops of strong hydrochloric acid being added after solution is complete. In using this stain the elastic fibers are treated with a few c.c. of the dye and then warmed for five minutes, after which the preparation is decolorized with acid alcohol.¹ The elastic tissue fibers will be stained a brownish violet by this process.

If the elastic tissue is very small in amount we usually resort to chemical means for the isolation of this material. Ten c.c. of sputum are mixed with an equal volume of 10 per cent. sodium hydrate solution and the mixture is boiled until it becomes homogeneous. Four volumes of water are then added, the entire mass well mixed, and either allowed to stand or to settle by centrifugation. In this way the constituents of the sputum are destroyed with the exception of the elastic tissue which has, however, become swollen and paler and does not have its characteristic appearance.

About 90 per cent. of cases showing elastic tissue in the sputum are of tuberculous origin, according to Dettweiler and Setzer. As the healing process begins and proceeds this elastic tissue gradually diminishes in amount, so that a constant presence or an increase in the amount indicates a progressive condition. It is seen in abscess of the lungs, in bronchiectasis, in pulmonary infarct, and occasionally in pneumonia. It is found in cases of gangrene of the lungs, although there are many statements that it is digested by the trypsin-like ferment so common in such conditions. Osler states that he has never seen a case of gangrene of the lung in which elastic tissue fiber could not be found.

(e) Crystals.

Crystals are never found in the freshly formed sputum, being indicative of stagnation of the material within the body or of decomposition after being expectorated.

(1) Fatty Acid Crystals.

These crystals occur most frequently in the sputum of gangrene, putrid bronchitis and of chronic tuberculosis. They occur as distinct needles, either singly or in groups, may be short and relatively thick with pointed ends, or they may be long and very closely resemble elastic fiber. Pressure upon the cover-glass will produce varicosities in these crystals which do not appear in the case of elastic tissue. They are soluble in alkalies and in ether and melt into fat globules if the slide be warmed.

(2) Cholesterin.

Crystals of cholesterin are found in the sputum of chronic lung abscesses, empyema and chronic tuberculosis. They are not as frequent as are the fatty acid crystals, but are usually associated with them when they are present. These crystals have a distinct rhomboid form with notched angles.

(3) Hematoidin.

Crystals of hematoidin occur very rarely in sputum, and then only when extravasation has taken place into the alveoli. They are found very rarely

¹ See Dargallo (Rev. Esp. de Med. y Cir., 1920, III, 588) (Abs. Jour. Am. Med. Assoc., 1921, LXXVI, 623) for another reliable stain for elastic fibers.

after direct hemorrhage unless the extravasated blood remains for some time in the alveoli. These crystals occur especially in abscess of the lungs, empyema, or perforating liver abscess. They are rhomboid or needle-shaped crystals, ruby-red in color, and may show small curved filaments projecting from the angles of the larger forms.

(4) **Leucin and Tyrosin.**

These substances are formed by the decomposition of protein material and are found, therefore, in the putrid sputum of an empyema, or from a perforating liver abscess, and in the very early discharges of true lung abscess. The tyrosin is found in the form of long refractive needles, frequently arranged in bundles, while the leucin appears as distinctly spherical masses with concentric striations and radiating lines.¹

(5) **Magnesium-ammonium Phosphate.**

These crystals appear in the sputum under the same condition as do the preceding tyrosin and leucin crystals. They are usually the typical coffin-lid crystals so common in decomposed urine, but may assume irregular structure.

(6) **Calcium Oxalate.**

These crystals occur in the sputum in conditions associated with decomposition and appear either in the typical octahedral crystal with a cross connecting the corners or as the more atypical dumb-bell shaped crystal.

(7) **Charcot-Leyden Crystals.**

These crystals are apparently derived from the eosinophile cells as they are more frequently present only in conditions in which the eosinophiles are very numerous, hence the term "leucocyte crystals." They increase in the sputum either from stagnation within the system or after being expectorated. They are associated in asthmatic attacks with the spirals of Curschmann, being frequently included in the meshes of the spiral.

These crystals form straight, pointed, colorless, hexagonal, double pyramids, resembling a very much elongated diamond. They have sharp elongated points with clear-cut edges, are very brittle, are colorless, show little refractility, and vary greatly in size. They may occur singly or in groups, forming either clusters or distinct Greek-cross types. They have been supposed to be identical with the spermin crystals of Böttcher, but the hexagonal type of the crystal as well as the facts that they do not show marked double refraction by polarized light and have but a single optical axis should serve to differentiate them from the spermin crystals. They are colored yellow with Florence's reagent and may be stained with the polychrome and other blood dyes. (See Semen.)

(f) **Bacteria.**

(1) **Saprophytes.**

The bacteria found in the sputum are very numerous and under normal conditions are purely saprophytic. We may even at times find many truly

¹ Pissavy and Monceaux (Bull. de la Soc. Méd. des Hôp. de Paris, 1922, XLVI, 376) find that tyrosin is constantly present in sputum of actively tubercular patients.

pathogenic organisms in the sputum which are of no clinical significance, although one may be led into making a diagnosis without sufficient clinical evidence.¹ These saprophytes may occur in the fresh sputum or develop therein after the specimen has stood for some time. The various chromo-



Katharine Hill

FIG. 3.—*Aspergillus fumigatus*.

genic bacteria are of particular interest as their development along the respiratory tract may so change the color of the sputum that the examiner may be led astray.

Among the ordinary saprophytes found in the sputum we find representatives of the streptothrix² and the leptothrix groups.³ Flexner and Warthin

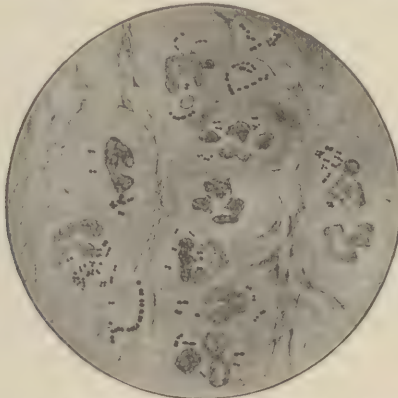


FIG. 4.—*Micrococcus catarrhalis*. (From Emery's "Clinical Bacteriology.")

and Olney have reported the presence of a streptothrix, the streptothrix episingeri, in the sputum of cases showing the clinical symptoms of pulmonary

¹ Boston (Interstate Med. Journ., 1914, XXI, 330) reports the relatively frequent presence of tubercle bacilli in the sputum of acute colds, with disappearance of these organisms during convalescence. Diphtheria bacilli are not infrequently found in the sputum, especially of "carriers." See Lippmann, Münch. med. Wchnschr., 1921, LXVIII, 772; Port, Ibid., 949; Singer, Med. Klinik, 1921, XVII, 1416; Meyer, Ibid., 1520.

² See Claypole, Jour. Exper. Med., 1912, XVII, 99; Bridge, Osler Memorial Volumes, 1910, I, 337; Glaser and Hart, Ztschr. f. klin. Med., 1921, XC, 294; Lenhart, Deutsch. Arch. f. klin. Med., 1921, CXXXVI, 129.

³ See Kato, Mitt. a. d. med. Fakultät der k. Univ., Tokyo, 1915, XIII, 441. Wherry and Oliver, Jour. Infect. Dis., 1916, XIX, 299.

tuberculosis. This condition is known as pulmonary streptothricosis. These organisms are about four times as thick as the tubercle bacillus; when stained they are resistant to decolorization by acids, but are slowly decolorized by strong alcohol. Stained specimens are easily made with the use of methylene-blue dyes or of Gram's method. The leptothrix group is particularly abundant in the mouth and is found in large numbers in the lungs in cases of pulmonary gangrene.



FIG. 5.—Budding forms of blastomycetes found in sputum.
(From photograph by W. A. Pusey.)

Yeast fungi occur in the sputum at times, but rarely in the fresh specimens.¹ They are oval or elliptical cells and are very refractive, sometimes resembling very closely fat droplets. Their appearance may vary from that of a simple oval cell without distinct limiting membrane to those with definite membrane and vacuoles. These cells are especially characterized by their tendency to throw out projections or buds at various points of their periphery. They vary in size from 1 to 40 microns in diameter. While these yeast fungi are usually extraneous, cases are reported (Busse) in which pathogenic yeasts

¹ See Breed, Arch. Int. Med., 1912, X, 108; Jour. Am. Med. Assn., 1913, LXI, 472.

have been found in anomalous pulmonary conditions. These organisms stain with the ordinary aniline dyes and appear in some instances to be acid-fast (resisting decolorization by acid). They are gram-positive.¹

Various types of molds are found in the sputum. Some of these appear to be distinctly pathogenic, while the majority are merely saprophytic. These molds are found in the true sputum only in cases associated with destructive processes of the lungs. There are at present many reports of cases showing that some of them at least may be distinctly answerable for primary infections.

Among these pathogenic molds we find certain types of the *Mucor*, of the 130 varieties of which six are known to be distinctly pathogenic. Besides this type we find, as the most important pathogenic mold of the sputum the *aspergillus fumigatus*, 16 cases of pulmonary affection (*pneumomycosis aspergillina*) having been traced by Sticker to this fungus.² Other types of the *aspergillus* are the *flavus*, *niger*, and the *sub-fuscus*. The *penicillium glaucum*³ and the *Oidium albicans* are occasionally found in the sputa.⁴

Among the bacteria which may be purely saprophytic we may find almost any of the pus-forming organisms. More frequently, however, when these organisms are present in excessive numbers a contamination or a direct pathogenic influence should be suspected. A form known as the *micrococcus tetragenus* occurs both as a pathogenic and a harmless organism. It consists, as its name implies, of four cocci arranged in a square within a mucous capsule. It stains with the ordinary dyes and is Gram-positive. This organism is found, in its pathogenic state, in bronchitis, tubercular cavities, and hemorrhagic infarctions.⁵ The harmless form differs from the pathogenic type in the fact that it cannot be cultivated.

The *sarcinae* are rarely found in the sputum. These organisms are somewhat smaller than those occurring in the stomach and are probably purely saprophytic in the sputum. They are found, however, in cases of putrid bronchitis, especially when this occurs in emphysematous lungs, in gangrene, tuberculosis, and pneumonia.

The *micrococcus catarrhalis* is found frequently in the sputum as a saprophyte, but may become pathogenic especially in some epidemics of la grippe.⁶ It is larger than the ordinary *staphylococcus*, while its arrangement in lateral pairs and its Gram-negative properties obscure its differentiation from the *gonococcus* and *meningococcus* unless cultural methods be employed. (See last chapter.)

In cases of general systemic blastomycosis, Eisendrath and Ormsby have

¹ See Henrici, Jour. Med. Research, 1914, XXX, 409. Simon, Am. Jour. Med. Sc., 1917, CLIII, 231.

² See von Teckman, Zur Lehre von der Pneumomycosis aspergillina, Inaug. Dissert., Basel, 1913. Ernst, Jour. Med. Research, 1918, XXXIX, 143; Müller, Deutsch. med. Wehnschr., 1920, XLVI, 1160; Kleberger, Ibid., 1170; Galdi, Rif. Med., 1921, XXXVII, 3; Mendelson, Jour. Am. Med. Assoc., 1921, LXXVII, 110; Farah, Presse méd., 1921, XXIX, 713; Assimis, Bull. Méd., 1921, XXXV, 960; Lapenta, New York Méd. Jour., 1921, CXIV, 620; Castellani, Douglas and Thomson, Jour. Trop. Med. & Hyg., 1921, XXIV, 150.

³ See Weidman, Proc. Path. Soc., Phila., 1915, XVII, 62. Magalhães, Brazil-Medico, 1916, XXX, 25.

⁴ See Nathan, Bull. Méd., 1920, XXXIV, 608.

⁵ Steele (Jour. Am. Med. Assn., 1914, LXII, 930) reports a case of general septicemia due to this organism.

⁶ Hastings and Niles, Jour. Exper. Med., 1911, XIII, 638; Clark and Murphy, Jour. Inf. Dis., 1921, XXIX, 306.

found the blastomycetes in the sputum. They recommend the examination of the unstained specimens after the addition of 10 per cent. sodium hydrate solution. In such preparations examined with a high-power dry lens, the typical refractile blastomycetes are observed. (See Parasitic Diseases.)

(2) Pathogenic Types.

(a) Tubercle Bacillus.

This organism is the most important pathogenic type found in the sputum. Its detection is usually easy and should be attempted in all suspicious cases, as an early diagnosis may frequently save the life of the patient. In the days before the organism was recognizable, physicians based their diagnosis of consumption upon the macroscopic appearance of the sputum. While such examinations frequently lead to a presumptive diagnosis of tuberculosis, nothing can settle the question except the microscopic examination of the sputum. This statement needs some modification in several ways. In the first place, specimens of tubercular sputum may not show the presence of the bacilli so that several examinations of sputum, collected at different periods, must be made. In the second place, the sputum may be examined in the very early stage of the disease and no tubercle bacilli be found, but in such cases the presence of elastic tissue fiber would be very significant of tubercular changes. In these days we have, fortunately, recourse to other diagnostic measures in case tubercle bacilli cannot be found in the sputum. I have reference here to the use of tuberculin, either introduced in the form of an injection as done by Koch, dropped into the eye as advocated by Calmette, as advised by Pirquet applied after the manner of vaccination, or used as an inunction as suggested by Moro.

In examining the sputum microscopically the fine cheesy particles previously mentioned are selected and smears of such material made upon glass slides. It is always advisable to make at least five such smears to insure definite results. Besancon and Biros¹ recommend the taking up of the fresh sputum on a platinum loop and the agitating of it, in turn, in several Petri dishes containing physiologic salt solution, in order to remove the saliva and extraneous mouth organisms, before making the smears. It has frequently been found that many suspicious-looking sputa show no tubercle bacilli when subjected to the ordinary methods of examination. In order, therefore, to increase the possibility of positive results, the sputum should be rendered homogeneous and fluid. The best method of accomplishing this is, in the writer's opinion, *Loeffler's*² *modification of the antiformin process of Uhlenhuth*. The technic is as follows: 5, 10, or more c.c. of the sputum are placed in a flask and mixed with an equal quantity of a 50 per cent. solution

¹ Bull. Acad. de Méd., 1921, LXXXV, 696.

² Deutsch. med. Wchnschr., 1910, XXXVI, 1987. See also, Krauss and Fleming, Jour. Lab. & Clin. Med., 1916, I, 919; Giraud et Derrien, C. R. soc. biol., 1916, LXXIX, 976; Suyenaga, Am. Rev. Tubere., 1919, III, 473; Goeckel, Med. Record, 1919, XCVI, 804; Greenfield and Anderson, Lancet, 1919, II, 423; Woolley, Jour. A. M. A., 1920, LXXIV, 525. Raphael and Eldridge (Jour. Am. Med. Assoc., 1920, LXXV, 245) recommend the treatment of 1 volume of the sputum with 2 volumes of Greenfield and Anderson's reagent (1 per cent. sodium carbonate in 1 per cent. phenol), shaking the mixture for 10 minutes, autoclaving for 20 minutes at 15 pounds pressure and then centrifuging the cooled mixture for 10 minutes. Decant the supernatant fluid and prepare smears from the sediment. See, also, Jones, Jour. Lab. & Clin. Med., 1920, VI, 41.; Gjorup and Bagger, Ugesk. f. Laeger, 1922, LXXXIV, 275.

of antiformin (a 10 per cent. solution of sodium hypochlorite containing 5 to 10 per cent. of sodium hydrate) and boiled for a period not exceeding 15 minutes. Solution occurs associated with considerable foaming and browning of the mixture. For every 10 c.c. of this solution are now added 1.5 c.c. of a mixture of 1 part of chloroform and 9 parts of alcohol. After thoroughly shaking to produce a fine emulsion, portions of the fluid are placed in sedimenting tubes, the tubes are corked, and centrifuged for fifteen minutes. The heavier elements collect in a film just above the chloroform, which film holds the tubercle bacilli owing to the marked affinity of chloroform for the fatty and waxy material in these organisms.¹ The supernatant liquid is poured off and the film above mentioned is removed and placed upon a glass slide, the excess of fluid being taken up with filter-paper. As a fixative a drop of egg-albumin, preserved with $\frac{1}{2}$ per cent. carbolic acid, is added and a thin spread made by means of a second slide. This smear is allowed to dry and is then stained by one of following methods. This enrichment process of Loeffler furnishes preparations which often show a remarkable increase in numbers of tubercle bacilli as compared with those found by the usual smear methods. A further advantage of this method is that practically all organisms, with the exception of those of the acid-fast type to which the tubercle bacillus belongs, are destroyed. If the mixture be allowed to stand for a few hours instead of being boiled, one may obtain material for pure-culture or for inoculation purposes, as the secondary invaders are eliminated. For these latter purposes it is necessary to wash the sediment frequently with sterile water by means of centrifugation and decantation to remove the excess of alkali present. This may, of course, diminish the number of tubercle bacilli and, at the same time, cause contamination of the specimen. Hence, inoculation and cultural experiments frequently give negative results.

A somewhat different method of eliminating the secondary invaders and of obtaining the tubercle bacillus in pure culture from the sputum (as well as other material) has been introduced by Petroff.² By the employment of a special culture medium to which gentian violet is added, Petroff takes advantage of the fact that this dye will inhibit the growth of many organisms but will not retard the development of the tubercle bacillus at the dilution at which it is used. Further, he adopts, as his homogenizing agent, an alkaline solution to which the tubercle bacillus is resistant while the viability of most other organisms is quite strongly affected.

Preparation of the Culture Medium

Five hundred grams of beef or, preferably, veal are infused in 500 c.c. of a 15 per cent. solution of glycerin in water. Allow the infusion to stand for 24 hours and squeeze the meat in a sterile press, collecting the juice in a sterile beaker.

¹ See Panzer, Ztschr. f. physiol. Chem., 1912, LXXVIII, 414; Wells, Interstate Med. Jour., 1914, XXI, 221. Bürger, Biochem. Ztschr., 1916, LXXXVIII, 155.

² Jour. Exper. Med., 1915, XXI 38; Bull. Johns Hopkins Hosp., 1915, XXVI, 275; also Mitchell and Simmons, Jour. Am. Med. Assn., 1915, LXV, 245; Williams and Burdick, Jour. Bacteriol., 1916, I, 411; Keilty, Jour. Exper. Med., 1915, XXII, 612 and Ibid., 1916, XXIV, 41; Lewis, Ibid., 1917, XXV, 441; Stewart, Ibid., XXVI, 755; Corper, Fiala and Kallen, Jour. Infect. Dis., 1918, XXIII, 267; Corper, Am. Rev. Tuberc., 1919, III, 461; Wilson, Brit. Med. Jour., 1920, I, 146; Griffith, Jour. Path. and Bact., 1920, XXIII, 120; Lyall, Amer. Rev. Tuberc., 1922, V, 893; Goodman and Moore, Jour. Inf. Dis., 1922, XXX, 58.

Sterilize the shells of one dozen fresh eggs by pouring hot water upon them. Carefully break the eggs into a sterile beaker and thoroughly mix the white and yolk with a sterile rod. Filter through sterile gauze into a sterile graduate.

To two parts of the egg add one part of the meat infusion and add to this mixture sufficient 1 per cent. alcoholic solution of gentian violet to make a dilution of 1 to 10,000. Tube about 5 c.c. of this mixture in each sterile test-tube and inspissate the slanted tubes for three successive days: on the first day at 85°C. until all the medium is solidified; on the second and third days for not more than 1 hour at 75°C. (If this medium is to be used for the isolation of the bovine type of tubercle bacillus, the glycerin may be omitted in making the meat infusion.)

Method

Equal parts of fresh sputum (about 5 c.c.) and 3 per cent. sodium hydrate solution are well shaken and placed in the incubator for 20 to 30 minutes until the sputum is fairly well digested. Neutralize this mixture to sterile litmus paper with normal hydrochloric or sulphuric acid. Centrifugalize at high speed for 10 minutes. Decant the supernatant fluid and spread the sediment over several slants of the culture medium. Incubate for 7 to 12 days.

The cultural characteristics may be more or less variable, some of the colonies being of small pin-point size, while others are large and flat. Some of the types may decolorize the medium, while others appear violet. Morphologically, the organisms may vary from cocci to long rods.

By this method of Petroff tubercle bacilli may be detected in a comparatively short time in cases which have shown repeated negative results by the usual microscopic methods, as well as in cases which have not yielded positive results with animal inoculation following the antiformin method. It is simple, practical and reliable. It is recommended as it tends to increase the possibility of diagnosis in obscure cases and may, even, supplant animal inoculation tests.

Staining Characteristics.

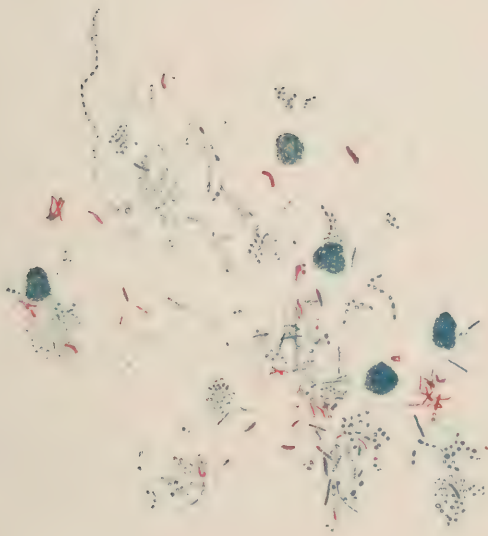
The methods of staining the tubercle bacillus depend upon the property, possessed by this organism, of taking up the aniline dyes with great difficulty, but, when once stained, of becoming just as resistant against decolorization. Breskman (Jour. Am. Med. Assn., 1910, LIV, 1591), Dixon (Ibid., 1913, LX, 993) and, more recently, Wherry (Jour. Infect. Dis., 1913, XIII, 144) have shown that the tubercle bacillus varies its acid-fast properties in its development on culture media, becoming more acid-fast or non-acid-fast according as the cultural conditions are or are not favorable for the synthesis of fat. Tamura (Ztschr. f. physiol. Chem., 1913, LXXXVII, 85) demonstrates that this acid-fast property is due to the presence in the bacterial cell of an alcohol, mykol ($C_{29}H_{56}O$), which is partially bound to some higher fatty acid in the form of an ester.¹

Ziehl-Neelsen Method.

The smears are made upon glass slides and are fixed by passing several

¹ See Goris (C. R. Acad. des Sc., 1920, CLXX, 1325) and Goris and Liot (Ann. Inst. Pasteur, 1920, XXXIV, 497) for studies of the chemical composition of the tubercle bacillus.

PLATE I.



Kultur no. 11.11

TUBERCLE BACILLI IN SPUTUM. ZIEHL-NEELSEN METHOD.

times through a flame. The smear is covered with carbol-fuchsin solution¹ (a mixture of 90 parts of 5 per cent. carbolic acid water and 10 parts of a saturated alcoholic solution of fuchsin), and is then heated over a flame for one to three minutes in such a way that the staining solution steams, but does not boil. If the staining solution is heated too strongly the smear decolorizes less readily so that it is very good practice never to boil the staining solution. Where several slides are to be examined, the writer has found the copper plate of Ehrlich very useful.² The slides are laid upon the plate, are covered with the stain, and allowed to heat for 10 minutes. The usual technic is, however, to heat a single slide at one time, more of the stain being added as the first evaporates. Some workers find that the immersion of the smear in cold carbol-fuchsin for 24 hours gives somewhat clearer pictures, but the time is too long for the ordinary laboratory diagnosis. Having thus stained the smear with the carbol-fuchsin solution, it is then decolorized. The tubercle bacillus is not only acid-fast, but also alcohol-fast, so that we use decolorizing agents containing both acid and alcohol. There are many acid-fast organisms known, such as the bacillus lepræ, the smegma bacillus, the timothy bacillus, the butter bacillus and many saprophytic bacilli found in water, soil, and manure.³ Few or none of these organisms are absolutely both alcohol and acid-fast, so that the use of the combined decolorizer will usually differentiate the tubercle bacillus. Many decolorizing agents have been advised, but the writer finds the use of a 10 per cent. solution of sulphuric acid in 95 per cent. alcohol very reliable. This decolorizing agent does not burn the specimen nor does it prevent the morphological characteristics from appearing in a clear-cut way. Some workers advise the use of a 2 per cent. hydrochloric acid in 80 per cent. alcohol, while others use 25 per cent. nitric acid, followed by alcohol.⁴ The technic of decolorization is as follows: Wash the smear, which has been stained with carbol-fuchsin, in water and flood the specimen with the decolorizing solution until only the faintest pink color is seen in the smear. It frequently happens that the thicker portions of the smear resist this decolorizing so that it may be necessary either to make a new smear or to examine merely the portions which have been decolorized. After decolorization is complete the specimen is washed in water and counter-stained with Löffler's methylene blue (saturated alcoholic solution of methylene blue 30 c.c., 100 c.c. of a 1 to 10,000 aqueous potassium hydrate solution) for a few seconds, after which the specimen is washed with water, dried between filter-paper, and examined with the oil immersion lens.

¹ See Verhoeff, Jour. Am. Med. Assn., 1912, LVIII, 1355. Cerqueira, Brazil-Medico, 1915, XXIX, 321; Klein, N. Y. Med. Jour., 1916, CIII, 217; Lesieur, Jacquet and Piutenet, C. R., soc. biol., Paris, 1919, LXXXII, 251; Kongsted, Centralbl. f. Bakt. u. Parasitenk., Abt. 1, 1920, LXXXIV, 513; Konrich, Deutsch. med. Wchnschr., 1920, XLVI, 741; Schultetigges, Ibid., 1225; Kieffer, Amer. Rev. Tuberc., 1921, V, 662.

² See Schmidt, Jour. Am. Med. Assn., 1915, LXIV, 823.

³ Lycopodium spores, cork cells, honey-comb wax and other substances not infrequently found in the sputum are acid-fast and may prove very misleading. See Sartory, Bull. l'Acad. Méd. Paris, 1919, LXXXI, 281, who calls attention to confusing bacteria belonging to the oospora group.

⁴ Bourdy (Bull. Sci. Pharmacol., 1918, XXV, 296) advises the use of 10 c.c. strong ammonia water in 90 c.c. of 95 per cent. alcohol. Keilty (Jour. A. M. A., 1916, LXVI, 1619) uses 30 per cent. hydrochloric acid without alcohol.

In such preparations the tubercle bacilli are seen as bright red rods, somewhat bent, sometimes much curved and occasionally showing distinct branching forms.¹ In a few preparations one may find the curves of the bacillus so marked that a very close resemblance to the spirillar forms obtains. These organisms occasionally show a distinct beading, giving the appearance of bright red cocci.² The size of these organisms varies from $1\frac{1}{2}$ to $3\frac{1}{2}$ microns and about $\frac{2}{10}$ microns in width. They may be single or arranged in clumps, sometimes in the form of distinct crosses, sometimes parallel, and very frequently forming acute angles by the joining of two bacilli.

Gabbet's Method.

This method is much more simple than the preceding, but is not as reliable. By this method the decolorization and counter-staining are carried out in one operation. The smears are prepared as previously described and stained with the carbol-fuchsin solution. The excess of the staining solution is drained off without washing and is replaced by Gabbet's methylene blue solution (methylene blue 2 grams, sulphuric acid 25 c.c., water 75 c.c.). This solution is allowed to act for one to three minutes and is then washed off with water and the specimen dried and examined. The tubercle bacilli will appear as bright red rods as previously described, while the other organisms as well as the various cellular types will be stained blue.

This method is not as reliable as is the former, owing to the fact that the alcohol-fast bacilli resist decolorization and may confuse one in making the diagnosis. Moreover, the use of the strong acid may cause decolorization of some of the tubercle bacilli and will, therefore, give rise to wrong ideas. These methods are the ones usually followed in routine laboratory work for the detection of the tubercle bacillus.

Pappenheim's Method.

The technic of this method is as follows: The preliminary staining is carried out with carbol-fuchsin solution as previously outlined. The specimen is then drained and covered with the decolorizing solution, which is made by dissolving 1 gram of rosolic acid in 100 c.c. of absolute alcohol, saturating the mixture with methylene blue and adding 20 parts of glycerin. This solution is drained off slowly and the process repeated several times. The slide is washed in water, dried between blotting-paper and examined with the immersion lens. The tubercle bacilli are stained red, the other organisms blue.

Much's Method.

As is well known, certain specimens of undoubted tubercular sputum as well as tissues frequently do not show tubercle bacilli when any of the above methods of staining are used, owing to the facts that neither is every acid-alcohol-fast bacillus the tubercle bacillus nor is every tubercle bacillus absolutely acid-fast. It is to Spengler³ and, more especially, to Much⁴ that we

¹ See Dixon, Jour. Am. Med. Assn., 1913, LX, 993 and 1294.

² See Babes, Berl. klin. Wchnschr., 1914, LI, 501.

³ Deutsch. med. Wchnschr., 1905, XXXI, 1228 and 1353; Ibid., 1907, XXXIII, 337.

⁴ Beitr. z. Klin. d. Tuberk., 1907, VIII, 85 and 357. See Meader, Am. Jour. Med. Sc., 1915, CL, 858; Miller, Jour. Path. & Bacteriol., 1916, XXI, 41.

are indebted for a method which will demonstrate these non-acid-fast types of tubercle bacilli, which are distinctly granular, frequently appearing as mere granules rather than as true bacilli. These granules, under certain unknown conditions, change into true bacillary types and *vice versa*.¹ Much's method is a modified Gram method, the tubercle bacilli being Gram-positive.² Prepare smears as described above. Cover this smear with a carbol-methyl violet solution (10 c.c. of a saturated alcoholic solution of Gruebler's methyl violet B. N. mixed with 90 c.c. of 2 per cent. aqueous carbolic acid solution) and heat to boiling several times. Wash off stain with water and cover smear with Lugol's solution (iodin 1 gram, potassium iodid 2 grams, water 300 c.c.) for 5 minutes. Wash with water and treat with 5 per cent. nitric acid for one minute and follow this with 3 per cent. hydrochloric acid for 10 seconds. Without washing place the slide in a mixture of equal parts of acetone and absolute alcohol until the smear is colorless. Wash with distilled water and counter-stain with 1 per cent. aqueous solution of safranin for a few seconds. Wash in water, dry thoroughly, and examine with the immersion lens. The tubercle bacilli and the granular forms appear bluish while the other organisms are red.³

Value of Examinations.

Brown⁴ in an able manner has summed up the value of the sputum examination for tubercle bacilli. He gives as his reasons for believing that one should be guarded in forming an opinion of the prognosis of certain cases the following points: (1) Many of the tubercle bacilli may not be stained at all. (2) Old foci may give off very few and young foci no bacilli at all. (3) By the occlusion of a bronchus the contents of a focus may be shut off entirely for a time and thus the expelled sputum may contain a large number of tubercle bacilli. (4) The organisms may be present one day and not again for months. (5) The organisms may be abundant in one part of a specimen and none be found in others. (6) Some patients with fatal tuberculosis (caseous pneumonia or acute miliary tuberculosis) may have no bacilli in the sputum, while in other cases the organisms are present even before physical signs obtain. (7) In severe cases with bronchitis the secretion of the bronchus will dilute the sputum and give the appearance of a reduction in the number of organisms.

While the number of bacilli in the sputum may thus vary, it is usually in direct ratio to the severity of the disease, although for the reasons above mentioned too much reliance should not be placed upon the number of organisms found. Brown recommends the use of a somewhat modified Gaffky's table in judging of the prognosis in any particular case. The cases are classified as follows, being designated by the Roman numerals:

¹ See Korber, *Deutsch. med. Wchnschr.*, 1912, XXXVIII, 1494; Weiner, *Münch. med. Wchnschr.*, 1914, LI, 1838; Brückner, *Ztschr. f. klin. Med.*, 1914, LXXX, 360; Mircoli, *Gazz. d. osp.*, 1914, XXXV, 617; Tallo, *Gazz. internaz. di med.*, 1915, XVIII, 241.

² See Benians, *Jour. Path. and Bacteriol.*, 1912, XVII, 199.

³ See Mas y Magro, *Rev. valenc. de cien. méd.*, 1913, XV, 37; Distaso, *Lancet*, 1920, I, 19.

⁴ *Montreal Med. Jour.*, 1901, XXX, 769; *Jour. Am. Med. Assn.*, 1903, XL, 514; Effler, *Ztschr. f. Tuberk.*, 1916, XXVI, 418.

- I. Only one to four bacilli in whole preparation.
- II. Only one on an average in many fields.
- III. Only one on an average in each field.
- IV. Two to three on an average in each field.
- V. Four to six on an average in each field.
- VI. Seven to twelve on an average in each field.
- VII. Thirteen to twenty-five on an average in each field.
- VIII. About fifty on an average in each field.
- IX. About 100 on an average in each field.

There has been some attempt to base a prognosis upon the form and grouping of the tubercle bacilli, the short rods indicating a rapid growth while the longer form shows a slower development.¹ The continued expectoration of large numbers of bacilli would indicate a cavity, while the sudden increase in numbers associated with an increase in the cellular elements would point to lung disintegration. A steady decrease over a long period of time would indicate improvement. It should be stated as a working rule that the finding of a single or a very few organisms in the sputum should be looked upon with suspicion, but that an absolute diagnosis should be made only after repeated examination has shown the presence of the tubercle bacilli.² The worker will find in the study of preparations stained as above that artefacts are very common.

The sputum in tubercular cases rarely shows tubercle bacilli in pure culture. One usually finds large numbers of streptococci,³ staphylococci, micrococci catarrhalis, and frequently influenza bacilli and pneumococci. Pus cells may be few or many. Blood-cells may or may not be present, while elastic tissue fiber is very frequent, appearing in many cases before tubercle bacilli can be demonstrated. The writer has frequently found sputa showing fairly large numbers of tubercle bacilli becoming practically negative if the sputum be allowed to stand exposed to the air for 48 hours. The explanation is that the other organisms so far outgrow the tubercle bacillus that they prevent any further development of this latter organism and bring about such degeneration that the staining qualities of the tubercle bacillus are markedly affected. This fact has been taken advantage of in the clinical use of injections of pus organisms into tubercular joints.

(b) *Lepra Bacillus.*

The bacillus lepræ, first described by Hansen, is a small slender bacillus from 4 to 6 microns in length and surrounded by a slimy envelope. These bacilli behave toward staining reagents very much like the tubercle bacillus, but are less resistant toward acid and alcohol than is the tubercle bacillus, so

¹ See Wilson, *Lancet*, 1914, II, 1198; Cohn, *Beitr. z. Klin. d. Tuberk.*, 1914, XXXI, 1; Kirchenstein, *Ibid.*, 33.

² The type of tubercle bacillus found in the sputum is practically always the human strain. Only seven cases are reported in the literature of infection with the bovine type. See Griffith, *Brit. Med. Jour.*, 1914, 1, 1171; Möllers, *Deutsch. Med. Wchnschr.*, 1914, XL, 1299. Wang, *Jour. Path. & Bacteriol.*, 1916, XXI, 14; Eastwood and Griffith (*Jour. Hyg.*, 1916, XV, 257) report 21 per cent. of bone and joint tuberculosis of the bovine type in a total of 261 cases. See, also, Griffith, *Lancet*, 1917, I, 216; Kendall, Day and Walker, *Jour. Infect. Dis.*, 1920, XXVI, 45 and 77; Cobbett *Lancet*, 1922, I, 979.

³ See Harvey, *Jour. Med. Res.*, 1917, XXXV, 279; Corper, Donald and Antz, *Jour. Inf. Dis.*, 1919, XXIV, 496; Lucke and Hague, *Ibid.*, 531.

that a differentiation is possible provided decolorization is rather severe. The stained bacilli often show clear spots or appear as if made up of stained granules.

These organisms may be found in many cases of leprosy in the sputum or nasal secretion, so that in doubtful cases a differentiation is necessary. While these organisms stain much more easily than do the tubercle bacilli and are more easily decolorized, it may be necessary to resort to inoculation experiments to make the absolute differentiation.¹

(c) **Smegma Bacillus.**

This bacillus may be found normally in the saliva, coating of the tongue, the tartar of the teeth, and in the crypts of the tonsils. Pathologically, it may be found in cases of simple bronchitis, in the sputum in cases of gangrene of the lungs, and in the suppurative discharges from the ears. While these organisms are much more commonly confused with the tubercle bacillus when the urine is examined than when the sputum is investigated, yet they must be borne in mind in every sputum examination. It may be necessary to resort to inoculation experiments to decide the question, but ordinarily the use of the differentiating stain of Pappenheim (previously described) as well as the following method of Bunge and Trantenroth may be used. After fixation of the smear, the fat is removed by soaking the specimen in absolute alcohol. The preparation is now covered with a 5 per cent. solution of chromic acid for 15 minutes, after which it is washed with water. The smear is stained with carbol-fuchsin, decolorized with 16 per cent. sulphuric acid for three minutes, and is then counter-stained for five minutes in a concentrated alcoholic solution of methylene blue. This method shows the tubercle bacillus as distinct red, while the smegma bacillus is blue. While both of these methods of differentiation are usually applicable, yet one occasionally finds the smegma bacillus resisting the action of 16 per cent. sulphuric acid for 30 minutes and of strong alcohol for 12 hours.²

(d) **The Timothy Bacillus.**

This organism is present in the mouth reaching it through the medium of butter and milk, which may contain it in large numbers. These bacilli re-

¹ See Duval, Jour. Exper. Med., 1911, XIII, 365; also Duval and Wellman, Jour. Am. Med. Assn., 1912, LVIII, 1427; Clegg cultivates this bacillus in symbiosis with amoebae and cholera vibrios (Philip. Jour. Sc., Sec. B., 1909, IV, 403); Smith, Lynch and Rivas have demonstrated the transmissibility of the lepra bacillus by the bed-bug (Am. Jour. Med. Sc., 1913, CXLVI, 671). See, also, Wolbach and Honeij, Jour. Med. Research, 1913, XXIX, 367; Honeij and Parker, Ibid., 1914, XXX, 127. Harris and Lanford, Jour. Med. Res., 1916, XXXIV, 157. Greenbaum and Schamberg (Jour. Am. Med. Assoc., 1922, LXXVIII, 1205) recommend the examination of the fluid obtained by aspiration of suspected nodules. The technic consists in the use of a syringe with tightly fitting plunger, a small record syringe by preference, a short pointed needle of average gage, and a few drops of salt solution or distilled water. The node is first gently massaged in order to bring as much fluid into it as possible. The needle is then introduced, after careful cleansing of the cutaneous surface, and the salt solution or distilled water slowly injected and withdrawn several times, thus making a sort of emulsion of the tissue about the needle point. The needle and its contents are then withdrawn, and smears are made from the fluid.

² Hartmann (Schweiz. med. Wchnschr., 1921, LI, 657) believes that there are two forms of smegma bacilli, one that can be cultivated and one that cannot.

sist the decolorizing action of both alcohol and acid to almost the same extent as do the tubercle bacilli, but they usually appear as somewhat longer and thicker rods. Strangely enough, this organism produces a lesion in guinea-pigs which resembles very closely that of true tuberculosis, so that the inoculation test will not always be conclusive unless other animals are inoculated with material from the first one, in which case no lesions develop in the later animals. Fortunately, the cultural peculiarities of this organism are markedly different from those of the tubercle bacillus, as the former develops readily on the ordinary culture media.

(e) **The Pneumococcus (*Diplococcus pneumoniae*).**

This organism, discovered by Fränkel and elaborated by Weichselbaum, is generally recognized as the etiologic factor in cases of acute croupous pneu-



FIG. 6.—*Diplococcus pneumoniae*. (Williams.)

monia, although other organisms not infrequently give rise to this condition. It is found in large numbers in the sputum and other exudates, appearing as a small slightly elongated conical or lance-shaped coccus, which shows a marked tendency to occur in pairs (diplococci) with the broader ends in apposition. Occasionally it is arranged in short chains resembling streptococci. In exudates and in the blood this organism is usually enveloped by a well-defined hyaline zone or capsule. In clinical work it is rarely necessary to stain this capsule to identify the organism but if this be desirable it is best done by the method of Rosenow.¹ It stains well with the ordinary dyes and is Gram-positive.

Typing of Pneumococci.

It has been found that active immunization of laboratory animals may be brought about by preliminary injections of attenuated or dead pneumo-

¹ Jour. Infect. Dis., 1911, IX, 1.

cocci followed by subsequent injections of gradually increasing doses of living virulent organisms. In the immune sera from such animals specific agglutinins were first thoroughly studied by Neufeld and since then by Wadsworth, Hess and others. In these studies it was found by Neufeld and Haendel that as regards their reactions toward immune serum several types of pneumococci exist. Later Dochez and Gillespie, studying isolated pneumococci by means of animal inoculation and by agglutination, divided the pneumococci into 4 groups. Groups I and II are made up of organisms that are respectively identical, as they react with sera produced by individuals of the same group, although Group II has been shown recently to contain several subgroups, IIa, IIb and IIx. Group III is a distinctive one and represents the pneumococcus (*streptococcus*) *mucosus*. Group IV is a mixed group of organisms which have no distinctive characteristics. Groups I and II seem to be the only ones which give exact serological reactions.

It has been found that Groups, I, II and III are most frequently associated with disease, while Group IV is often found in the mouths of normal people. Precipitins, as well as agglutinins, have been found in the pneumococcus immune serum, the organisms used in these tests being brought into solution either with bile or salt solution. By immunization of horses with the various types of pneumococci, a serum for Type I and Type II has been produced, which offer much hope in the treatment of this disease. As the use of the proper serum depends entirely on the identification of the type of the invading organism, methods have been introduced for this work, which enable the investigator to "type" the sputum of the infected individual in a short time. The percentage of incidence of infection with these groups is about as follows, taken from the average of different workers: Group I, 33 per cent.; Group II, 33.5 per cent.; Group III, 12.5 per cent.; and Group IV, 20 per cent. Dochez and Avery state that these groups vary in the order of their virulence as follows: Group III, II, I, and IV. The degree of protective power developed in the sera of animals immunized against members of the different groups varies inversely with the virulence and with the amount of capsular development of the organisms.

The usual method of typing these organisms is as follows: The patient is instructed to wash out his mouth with some mild antiseptic solution and to expectorate the sputum obtained by coughing, into a Petri dish. This is not always an easy matter as many cases of pneumonia show very little sputum that can be raised. The sputum is then washed several times in sterile salt solution contained in a series of small watch glasses or dishes. It is then rubbed up in a mortar with a little ($1\frac{1}{2}$ to 1 c.c.) of salt solution or bouillon and injected intraperitoneally into a white mouse. The pneumococci develop in the peritoneal cavity and after 8 hours the animal is killed and the peritoneal cavity washed with salt solution or bouillon (5 to 6 c.c.). The washings are then placed in a centrifuge tube and whirled at a low speed for several minutes in order to throw down the leucocytes. The supernatant fluid is then withdrawn and centrifuged at a high speed and the organisms thus obtained are suspended in fresh salt solution. This suspension is then

added to equal parts of the sera of Types I and II, in separate tubes, in dilutions of 1:2.5 and 1:10, in order to note the precipitin reaction.

Several modifications of the above method and several new methods of typing have been introduced. We give here that of Oliver as it is rapid and checks well with other more complicated methods. From 1 to 2 c.c. of sputum are placed in a clean centrifuge tube and to it are added 3 to 5 drops of undiluted ox bile (or a 10 per cent. solution of sodium taurocholate) and a sufficient quantity of sterile physiologic salt solution to insure a specimen of sufficient fluidity to permit of centrifugation. The mixture is then thoroughly stirred and broken up with a glass rod. It may prove advantageous to do this mixing by grinding in a small mortar with a pestle. The tube is then heated in a water bath at a temperature of 42 to 45°C. for 20 minutes; after which it is centrifuged. Of the centrifugate 0.3 to 0.5 c.c. quantities are carefully pipetted into each of 3 small scrupulously clean tubes. To the first tube is added 1 to 2 drops of undiluted Type I pneumococcus antiserum, and to the second and third tubes the same quantity of Type II and Type III antiserum respectively. A positive precipitin test is evidenced by an almost immediate clouding and flocculation, which is increased by heating at 42°C. in a water-bath for from 10 to 20 minutes.¹



FIG. 7.—Friedländer's bacillus (above); pneumococcus (below) (Greene).

Friedländer's Bacillus (*bacillus mucosus capsulatus*) is occasionally found in some cases of lobar and lobular pneumonia and occasionally may be considered the etiologic factor in such conditions, although it is usually a secondary invader.² These bacilli grow readily on artificial media, are encapsulated and stain easily with the ordinary dyes but are Gram-negative.

(f) The Influenza Bacillus.

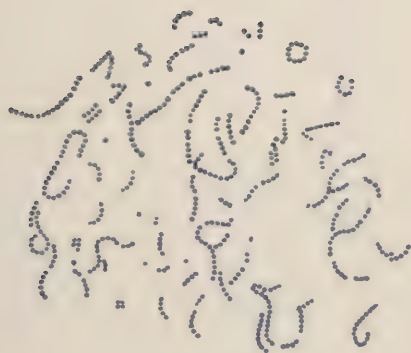
This bacillus, known as Pfeiffer's bacillus, is found in the bronchial sputum, especially in the pulmonary type of this disease.³ The most characteristic sputum is greenish-yellow

¹ Neufeld and Haendel, *Arb. a. d. Kais. Gesund.*, 1910, XXXIV, 293; Dochez and Gillespie, *Jour. Am. Med. Assoc.*, 1913, LXI, 717; Cole, *Arch. Int. Med.*, 1914, XIV, 50; New York Med. Jour., 1915, CI, 1 and 59; Dochez and Avery, *Jour. Exp. Med.*, 1915, XXI, 114; Lyall, *Ibid.*, 146; Dochez and Avery, *Ibid.*, XXII, 105; Avery, *Ibid.*, 804; Mathers, *Jour. Inf. Dis.*, 1915, XVII, 514; Wollstein and Benson, *Am. Jour. Dis. Child.*, 1916, XII, 254; Stillman, *Jour. Exp. Med.*, 1916, XXIV, 651; Hartman and Lacy, *Jour. Am. Med. Assoc.*, 1917, LXIX, 2165; Blake, *Jour. Exp. Med.*, 1917, XXVI, 67; Mitchell and Muns, *Jour. Med. Res.*, 1918, XXXVII, 339; Stillman, *Jour. Exp. Med.*, 1919, XXIX, 251; Loewe, Hirschfeld and Wallach, *Jour. Am. Med. Assoc.*, 1919, LXXIII, 170; Sailer, Hall, Wilson and McCoy, *Arch. Int. Med.*, 1919, XXIV, 600; Meyer, *Jour. Am. Med. Assoc.*, 1920, LXXV, 1268; Ferry and Blanchard, *Jour. Lab. & Clin. Med.*, 1920, VI, 23; Oliver, *Jour. Inf. Dis.*, 1920, XXVII, 310; *Ibid.*, 1921, XXIX, 518; Lord and Nye, *Jour. Exp. Med.*, 1921, XXXIV, 199, 201, 207 and 211; Thomsen and Christensen, *Acta Med. Scand.*, 1921, LIV, 501; Malloch, *Quart. Jour. Med.*, 1922, XV, 103.

² See Sisson and Thompson, *Am. Jour. Med. Sc.*, 1915, CL, 713; Sisson and Walker, *Jour. Exper. Med.*, 1915, XXII, 747.

³ See Davis (*Jour. Am. Med. Assn.*, 1915, LXIV, 1814) for a discussion of the rôle of this organism, as well as other members of the hemoglobinophilic group as secondary invaders in respiratory infections. Also, Wollstein, *Jour. Exper. Med.*, 1915, XXII, 445; Pritchett and Stillman, *Jour. Exper. Med.*, 1919, XXIX, 259; Winchell and Stillman, *Ibid.*, XXX, 497; Arnold, *Jour. Lab. and Clin. Med.*, 1920, V, 652; Loewe and Zeman, *Jour. Am. Med. Assoc.*, 1921, LXXVI, 986; Pilot and Pearlman, *Jour. Inf. Dis.*, 1921, XXIX, 47, 51 and 55; Meyer, Pilot, and Pearlman, *Ibid.*, 59; Pilot, *Ibid.*, 62; Olitsky and Gates, *Jour. Exp. Med.*, 1921, XXXIII, 125, 361, 373, and 713; *Ibid.*, XXXIV, 1; *Ibid.*, 1922, XXXV, 1.

PLATE II.



Katharine Hill

STREPTOCOCCUS PYOGENES. (METHYLENE BLUE STAIN.)

in color with lumps of pus in nummular form. The organisms are found in such sputum as small, short bacilli measuring $\frac{2}{10}$ to $\frac{3}{10}$ micron in breadth by $\frac{5}{10}$ micron in length. They usually occur singly, but may form chains. In the stained specimens these organisms show distinct polar stainings, appearing frequently as diplococci. They are stained with dilute carbol-fuchsin solution, faintly with the ordinary methylene blue solution, or are identified by their Gram-negative characteristics. The best counterstain used in the Gram method is either Bismarck brown or safranin, the organisms appearing both intra- and extra-cellular.

(g) The Bacillus Pertussis.

This organism, discovered by Bordet and Gengou, and elaborated by Klimenko, has been frequently found in the sputum in cases of whooping-cough. It resembles very closely the influenza bacillus, appearing as short, plump, ovoid bacilli, with rounded ends and lying singly or in small groups between the pus and epithelial cells. It stains feebly with the usual dyes often showing bipolar staining and is Gram-negative. This organism is rarely intra-cellular and may thus be distinguished from the influenza bacillus.

(h) Typhoid Bacillus.

This organism has been found in the sputum in typhoid fever cases showing a coexistent bronchitis or pneumonia. The sputum is usually hemorrhagic in character and shows the bacilli as short, thick rods, staining with the ordinary dyes and negative to Gram's stain.

(i) Staphylococcus and Streptococcus Pyogenes.

These organisms are found in practically every sputum examined and can be identified only by the use of cultural methods. They stain well with any of the aniline dyes and are Gram-positive. Their presence in the sputum has little pathologic significance.¹

(j) The Bacillus Pestis.

This bacillus of bubonic plague was discovered by Kitasato and Yersin in 1894. It is a short, thick bacillus, measuring from 0.8 to 2 microns in length and from 0.4 to 0.8 micron in thickness. A capsule may be usually made out and the stained organism frequently resembles a diplococcus, owing to the intense polar staining with intermediate faint staining. It is Gram-negative.

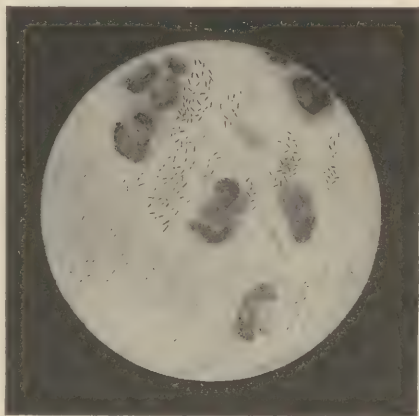


FIG. 8.—Bacillus influenza in sputum.
(Abbott.)

¹ The streptococcus viridans is not infrequently found in association with infections of the upper respiratory tract. See Cecil, Arch. Int. Med., 1915, XV, 150. Luetscher (Ibid., 1915, XVI, 657) discusses the importance of cultural methods in non-tubercular infections.

The bacillus pestis is found in the sputum of persons suffering from the pneumonic type of this disease and should be recognized owing to the markedly infective character of the material.¹ It may be necessary absolutely to identify the organism by inoculation and cultural experiments.

(k) **The Bacillus Anthracis.**

The sputum of cases of pulmonary anthrax may contain large numbers of these bacilli. These organisms are from 5 to 10 microns in length and from 1 to 1½ in breadth. They are frequently grouped in long segmented threads, the segments varying in length, but usually being two or three times as long as broad. Occasionally these bacilli may be single, but are usually multiple. They form oval spores in the middle of the short segments. The organism stains with the ordinary dyes and is also Gram-positive. For absolute identification cultural and inoculation experiments, the latter into

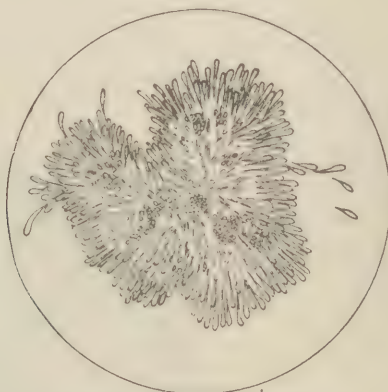


FIG. 9.—Actinomyces. (Williams.)

white mice, may be necessary, but the morphological characteristics will usually identify it.

(l) **The Bacillus Mallei.**

This organism of glanders is found in the sputum in the pulmonary form of this disease. Morphologically, there is nothing characteristic in the appearance of this organism beyond the fact of the presence of faintly staining areas in the protoplasm of the rather long bacilli. These organisms stain by Gram's method as well as with the ordinary aniline dyes. For a final diagnosis inoculation into a guinea-pig should be made.

(m) **Actinomyces Hominis (Ray Fungus).**

This fungus, which gives rise to the condition known as lumpy jaw in cattle, occasionally infects man, causing pulmonary conditions designated streptothricosis. The mucopurulent sputum in such conditions contains elastic tissue and small sulphur-yellow granules which are visible to the naked

¹ See Strong and Teague, Philip. Jour. Trop. Med., 1912, VII, 187; Williams, Jour. Am. Med. Assoc., 1920, LXXV, 370.

eye and are the characteristic findings of such cases. Macroscopically these granules are yellowish, grayish, or brownish in color, and are sometimes abundant and sometimes scarce.¹ They are very friable, and when gently crushed beneath the cover-glass and examined microscopically appear to have broken up into hyaline rounded masses at the margins of which, on close inspection, fine radial striations or filaments or hyaline club-shaped bodies, all closely set together, may be seen. The club-shaped bodies are variable in size and are composed of a hyaline refringent substance. In

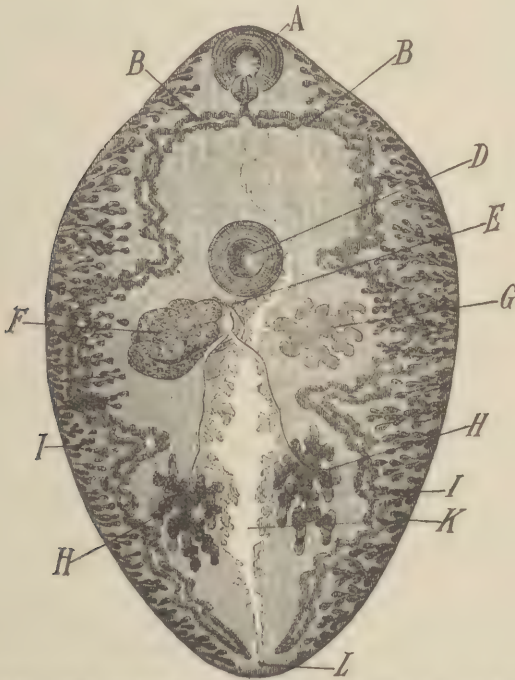


FIG. 10.—*Paragonimus westermanii* (ventral view); 10 × 1. A, oral sucker; B, ceca; D, acetabulum; E, genital pore; F, uterus; G, ovary; H, testes; I, vitelline glands; K, excretory canal; L, excretory pore. (Tyson after Braun.)

the granules obtained from the lesions in man the club-shaped formations are much less frequently observed than those obtained from the lesions in cattle (Mallory and Wright). If cover-glass preparations be made and stained with Gram's method, one will usually find isolated and matted filaments, many of which may be seen to branch, in addition to longer and shorter fragments of filaments and fine detritus of the same. If clubs are present in the granules they may be found scattered throughout the preparations. This organism grows readily on most media, nutrient broth to which

¹ Davis (Jour. Infect. Dis., 1914, XIV, 144) calls attention to actinomyces-like granules found in the crypts of tonsils and composed of bacilli, streptococci and spirilla. See, also, Rullmann, Münch. med. Wchnschr., 1914, LXI, 1899; Claypole, Arch. Int. Med., 1914, XIV, 104; Davis, Ibid., I; Cope, Brit. Jour. Surg., 1915, III, 55. Pilot and Davis, Jour. Infect. Dis., 1918, XXIII, 562; Waksman, Jour., Bacteriol., 1919, IV, 307.

a few drops of fresh human blood have been added being especially serviceable.¹

(g) **Animal Parasites.**

(a) **Amebæ.**

Artault has described a unicellular ameboid body which resembles very closely a leucocyte when stained, but, while motile, differs in refractility and staining quality. This he calls the ameba pulmonalis. In cases of perforating liver abscess the true amebæ coli may be found in the sputum, and, according to Flexner, in cases of abscess of the jaw communicating with the mouth.² It should be noted here that these organisms may not be numerous so that many slides may have to be examined.

(b) **Flagellates.**

Flagellated organisms, such as the trichomonas pulmonalis and the cercomonads, are found in the sputum associated with Dittrich's plugs in cases of gangrene, putrid bronchitis, and tubercular cavity formation. The trichomonas is probably identical with the trichomonas vaginalis or intestinalis.³

(c) **Cestodes.**

Not infrequently the lung is the seat of infection with the tænia echinococcus.⁴ In such cases various foreign bodies, such as fragments of membranes, scolices, hooklets, and cysts, may be found in the sputum. Such formations may, also, be found in cases of liver abscess perforating into the lung. Any one of the above formations is characteristic of this condition. The parasite will be discussed fully in the section on Feces, to which the reader is referred.

The sputum in such cases is usually purulent or mucopurulent and may be tinged with blood. This sputum may be expectorated over a long period of time and may even contain tubercle bacilli from a coexistent tuberculosis. A distinguishing point between pulmonary echinococcus disease and perforating liver abscess is that the sputum in the latter is usually bile stained.⁵

(d) **Trematodes.**

The most common of this class of animal parasites is the ordinary "lung fluke," which has been called also distoma pulmonale, distoma Westermanii distoma Ringeri, and Paragonimus Westermanii.⁶ The eggs of this parasite are much more frequently found in the sputum than are the parasites them-

¹ See Bridge, Jour. Am. Med. Assn., 1911, LVII, 1501; also, Schlegel, Kolle and Wassermann's Handb. d. path. Mikroorg., 1912, V, 301; Breed and Conn. Jour. Bact., 1910, IV, 585; Gordon, Brit. Med. Jour., 1920, I, 435; Colebrook, Brit. Jour. Exp. Path., 1920, I, 197; Henrici and Gardner, Jour. Inf. Dis., 1921, XXVIII, 232, Sanford and Magath, Minnesota Med., 1922, V, 71.

² These are probably identical with the endamebæ gingivalis found in the mouth in cases of pyorrhæa. (See next chapter.)

³ See Ohira and Noguchi, Jour. Exper. Med., 1917, XXV, 341.

⁴ See Filia, Pensiero med., 1914, IV, 741; also, Ortali, Gazz. d. osp., 1915, XXXVI, 225.

⁵ See Maliwa, Münch. med. Wchnschr., 1914, LXI, 2367.

⁶ See Nakagawa, Jour. Exper. Med., 1917, XXVI, 207; Jour. Parasitol., 1919, VI, 39.

selves, so that the diagnosis will rest with the finding of these ova. These eggs measure from 80 to 100 microns in length and 40 to 60 microns in width. They are brownish in color, oval in shape, have a smooth thin shell and a lid near one end which is quite characteristic. The parasite is from 8 to 10 mm. long, 4 to 6 mm. wide, and is very markedly rounded anteriorly, being nearly as thick as broad.

The sputum in such cases is usually small in amount, is very tenacious, and is reddish or rusty due to admixture of blood with the mucus. Frequently no blood is found, in which case the sputum will still be of a yellowish or brown color due to the eggs themselves. The sputum, also, contains many spirals, which resemble very closely the Curschmann spiral and, also, the Charcot-Leyden crystals.¹

The eggs of another species of distoma, the distoma hematobium, has been found in the sputum by Manson. Gage² has reported the finding of the larvæ of strongyloides intestinalis in the sputum.

V. THE SPUTA IN DISEASE

(1) Pulmonary Tuberculosis.

It has been truly said by Brown that pulmonary tuberculosis has no characteristic form of sputum. The amount voided may vary from the very slight type of fibroid tuberculosis to the very abundant sputum of cavity formation. It is to be said that the amount of pus will usually depend upon the extent of the secondary infection, although caseous degeneration may lead to the expectoration of large amounts of material resembling pus.

In the early cases of pulmonary tuberculosis we may find a small amount of sputum which is expectorated only in the morning. This may be very tenacious and resemble very much the sago-like sputum previously mentioned. Sooner or later depending upon the extension of the disease, there will appear small caseous particles which are very suggestive. As ulcerative processes proceed, the sputum becomes more profuse, yellowish or greenish in color, and muco-purulent in character. In any stage of this ulcerative tuberculous condition we may find blood in amounts ranging from a few blood-cells to a sputum loaded with blood from a hemorrhagic focus. Likewise we will find elastic tissue in more or less amount and tubercle bacilli varying from a few to many in each field. The color of the tubercular sputum may range through all the shades of the spectrum, the greenish shade being associated with a most marked decomposition. As stated previously, the most suspicious looking sputa frequently contain no tubercle bacilli.

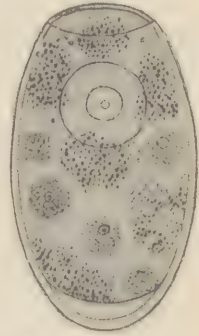


FIG. 11.—Ovum of *paragonimus westermani*, from sputum: 1000 \times 1. (Tyson after Braun.)

¹ For the mode of infection in these cases see Nakagawa, Jour. Infect. Dis., 1916, XVIII, 131, and Yoshida, Jour. Parasitol., 1916, II, 111.

² Arch. Int. Med., 1911, VII, 561. The larvæ of *ascaris lumbricoides* may cause definite pulmonary symptoms as shown by Ransom, Jour. Am. Med. Assoc., 1919, LXXIII, 1210. See also, Steiner, Schweiz. med. Wchnschr., 1920, L, 334.

(2) Croupous Pneumonia.

The early sputum of acute lobar pneumonia is usually yellowish-red in color and very tenacious in consistency. In some cases the sputum is mucoid and abundant for a few days, but soon takes on the characteristic reddish color from the presence of unchanged red blood-cells. Its consistency is so great that the receptacle may be inverted without allowing any material to run out. The characteristic rusty sputum, which is found when the exudation into the alveoli is taking place, is homogeneous, glairy, very tenacious, and deep red in color. This rusty sputum, while characteristic of pneumonia, is sometimes replaced by one ranging in color from a yellow to a green. These colors are due to different oxidation products of hemoglobin, and are, perhaps, more frequently observed in the stage of resolution when the sputum becomes less tenacious and more abundant. The greenish sputa in pneumonic conditions have some importance. This coloration may be due to a coincident jaundice or may arise from delayed resolution, especially when the exudate has been particularly hemorrhagic. It is, moreover, sometimes an indication that a true tubercular condition has intervened and, hence, that the prognosis must be guarded.

The so-called prune-juice sputum usually indicates a severe type of the disease, while at times it may signify merely a beginning resolution.¹

Fibrinous coagula are found, according to Osler, in every case in which search is made. These may vary from very small bronchial casts to very large branching types. Curschmann's spirals as well as the Charcot-Leyden crystals are frequently observed. The characteristic organism of this condition, the diplococcus lanceolatus of Fränkel, is usually found, but has only incidental importance, unless the type be determined, as it is so frequently present in the sputum of normal individuals.²

(3) Bronchopneumonia.

The sputum of this disease is rarely characteristic. It partakes of both the type of a bronchitic and a pneumonic sputum. It may, therefore, contain much mucus and pus, may be viscid, may be streaked with blood, but is rarely so distinctly rusty as in the croupous type of this disease. As the disease is so limited in extent it is more or less rare to find an abundant sputum or to observe fibrinous coagula. Microscopical examination shows various organisms, but nothing diagnostic.

(4) Acute Bronchitis.

The sputum in this condition is very scanty in the early stages, is usually very tenacious and is expelled with difficulty. This early bronchitic sputum is known as "*sputum crudum*" and consists of practically pure mucin, con-

¹ Pacini (Interstate Med. Jour., 1912, XIX, 536) has advanced the following reaction as characteristic of early pneumonic sputum: Mix 1 volume of sputum with 10 volumes of distilled water, agitate for 5 minutes and filter. To a test-tube containing 10 c.c. of distilled water add 5 drops of a 1 per cent. aqueous methyl-violet solution. Add to this latter 10 drops of the sputum filtrate. A positive reaction is shown by the appearance of a distinct red color.

² See Rosenberger and Dorworth, New York Med. Jour., 1913, XCVII, 532.

taining within its meshes a few leucocytes, red cells, bronchial epithelial cells, and a few myelin drops.

After a few days the sputum is increased in amount, becomes less viscid, and assumes the type of a distinct muco-purulent sputum. This sputum, called the *sputum coctum*, contains numerous pus-cells, is yellow or yellowish-green in color, shows the presence of large numbers of red cells, as a rule, and an increase of the polynuclear leucocytes over the mononuclear form. These mononuclear forms are more characteristic of the sputum of true tubercular conditions. Fat may be found, either in isolated drops or in large masses. As improvement in the condition occurs, the sputum becomes more abundant, and more distinctly purulent, and then gradually diminishes until it ceases. The sputum of acute bronchitis may give much information as to the course of the disease, as the transition from the viscid mucoid sputum through the abundant purulent stage to the final cessation is quite characteristic.

(5) Chronic Bronchitis.

(a) Simple Chronic Bronchitis.

In most of these cases the sputum is either very little in amount or is much more abundant than in the acute forms. Such cases of simple chronic bronchitis are usually those following the acute type of the disease in which we find the expectoration, for long periods of time, of a tenacious, viscid, and scanty sputum. Later it may become more abundant and muco-purulent and may have a dark color and a distinctly foul odor.

In the type of chronic bronchitis associated with cardiac disease we find large amounts of blood which may be fresh or changed, giving the typical prune-juice appearance. In such sputum we frequently find large numbers of the so-called "heart-disease cell" which have been previously described.

(b) Putrid Bronchitis.

This condition is brought about by dilatation of the bronchial tubes following a chronic bronchitis. The sputum lies stagnant in these dilated bronchi so that it decomposes to a great extent. The sputum in such cases is very abundant, is of an ash-gray or brown color, is markedly purulent, and has a very disagreeable odor. On standing it separates into the three layers which have been previously discussed. In such conditions no elastic tissue fiber is found, so that we have here a differentiation from gangrenous or tuberculous pulmonary conditions. The sputum in this condition is very similar to that found in bronchiectasis, which is usually associated with decomposition of the sputum. Whether a diagnosis is possible between a straight putrid bronchitis and bronchiectasis is doubtful, if one relies merely upon the sputum. The sputum in bronchiectasis occurs usually in the morning and is then very profuse. It shows, however, the characteristics of the sputum of putrid bronchitis, but is more commonly associated with the presence of pus, while 50 per cent. of cases show more or less profuse hemorrhage.

(c) Fibrinous Bronchitis.

The chief characteristic of this condition is the expectoration of more or less perfect bronchial casts. These may be single or may be distinctly branching, showing the arrangement of the entire bronchial tree. This condition

occurs quite frequently associated with many febrile diseases, but in the discussion at this point we have reference to the idiopathic type of the disease. The sputum in this latter class of diseases is mucoid and very abundant in the earlier stages. After a few days there is expectorated, following a severe coughing spell, a bronchial cast. This expectoration is usually tinged with blood. Such casts may be expectorated over long periods of time and their form may vary as previously described.

(6) **Bronchial Asthma.**

The sputum in bronchial asthma is, perhaps, more characteristic than that of any other pulmonary condition. During the paroxysm of asthma there may be no sputum, or it may be scanty, consisting of the glairy mucoid plugs known as the pearls of Laennec. The sputum contains many eosinophile cells and many alveolar epithelial cells with myelin degeneration. The mucoid sputum usually contains large numbers of the spirals of Curschmann along with the Charcot-Leyden crystals. In some cases of asthma one finds small cylindrical casts of bronchi. Some of these branch while the majority are straight and may taper at one end into the central fiber of a true spiral. Koessler and Moody (Jour. A. M. A., 1915, LXIV, 1104) report the presence in the sputum of asthmatics of a fusiform anaerobic bacillus together with pneumococcus, streptococcus hemolyticus and streptococcus viridans.¹

(7) **Influenza.**

The sputum of the pulmonary type of this condition shows in the early stages as a very scanty tenacious expectoration. Later it increases in amount, becomes muco-purulent and often blood-streaked, and is greenish-yellow in color. This sputum contains large numbers of Pfeiffer's bacilli, which have been previously discussed.

(8) **Gangrene of the Lung.**

The sputum in this condition is very profuse, is greenish-brown in color, is very offensive in odor, and is extremely fluid in character. It contains shreds of elastic tissue which serve to distinguish it from the sputum of putrid bronchitis or bronchiectasis. This sputum separates, as do other forms of sputum which have undergone stagnation and decomposition, into three distinct layers.

Microscopic examination shows fragments of necrotic tissue varying from very minute particles to those several cm. in length. Very few epithelial cells or leucocytes are found, but red blood-cells are more or less frequent. The bacterial content is usually very high, but nothing characteristic is found among these organisms.

(9) **Abscess of the Lung.**

The most characteristic feature of true abscess of the lung or of liver abscess which has perforated into the lung is the sudden appearance of a large amount of pure pus containing fragments of lung tissue. This material usually has the normal odor of pus, but may become offensive, although never as markedly so as in gangrene or putrid bronchitis. The sputum of the perforating liver abscess is usually distinguished from that of the true lung abscess by the so-called "anchovy-sauce" appearance. The color may

¹ See Walker and Adkinson, Jour. Med. Res., 1917, XXXV, 391; Ibid., 1920, XLI, 457.

vary, due to the presence of various types of bile pigment. Microscopically, bilirubin crystals may be found.

(10) **Perforating Empyema.**

The sputum of such conditions is composed almost entirely of pus and is thin and liquid. It contains many hematinoid crystals, but very little elastic tissue fiber or other tissue fragments. The odor is usually described as that of old cheese in the beginning, but soon becomes offensive owing to decomposition.

(11) **Pneumonoconioses.**

The sputum in these various conditions will depend upon the pigment with which the lung has been infiltrated. The expectoration is usually mucopurulent, very profuse, and is laden with coal-dust (anthracosis), iron-dust (siderosis), with stone-dust, chalk-dust or plaster of Paris (chalicosis), and with starch granules (amylosis).

(12) **Bronchial Spirochetosis.**

In 1906 Castellani reported the occurrence, in the island of Ceylon, of a form of bronchial infection caused by a spirochete, to which he gave the name of *spirochaeta bronchialis*. This organism appears to be polymorphous, is from 4 to 30 microns long and 0.2 to 0.6 microns in thickness, and shows from 2 to 8 or more undulations and its extremities of variable shape. It is actively motile but soon loses this motility after the sputum is expectorated. It stains quite readily with the usual basic aniline dyes, various polychrome methylene blue stains, or Fontana's spirochete stain, but it is negative to Gram's stain.

Since the discovery of this condition, various observers in various parts of the world have reported such cases, so that the disease appears to be very wide spread. It is usually confused with influenzal bronchitis, certain types of pneumonia and tuberculosis. It may occur in acute, sub-acute, or chronic form, the patient complaining of a cough with scanty sputum containing blood in more or less amounts. Recognition of the disease is made through a microscopic examination of the sputum collected after cleansing the mouth and throat. This sputum is mucoid, later becoming purulent and may be of a pinkish, reddish, or greenish-yellow color. In the cases reported by Nolf, it appeared to be extremely fetid, a characteristic not mentioned by Castellani or other observers. The sputum may be examined in the fresh state with the dark field illuminator or it may be stained by the stains mentioned above.¹

¹ Castellani, *Lancet*, 1906, I, 1384; Branch, *Brit. Med. Jour.*, 1906, II, 1537; Waters, *Tr. Soc. Trop. Med. and Hyg.*, 1909, p. 145; Phalen and Kilbourne, *Report of U. S. Army Board for Study of Tropical Diseases*, 1909; Tothwell, *Jour. Am. Med. Assoc.*, 1910, LIV, 1876; Chamberlain, *Philippine Jour. Sci.*, 1911, VI, 489; Chalmers and O'Farrell, *Jour. Trop. Med. & Hyg.*, 1913, XVI, 320; Taylor, *Ann. Trop. Med. & Parasitol.*, 1914, VIII, 13; Harper, *Jour. Trop. Med. & Hyg.*, 1914, XVII, 194; Fantham, *Ann. Trop. Med. & Parasitol.*, 1915, IX, 391; Lurie, *Jour. Trop. Med. & Hyg.*, 1915, XVIII, 269; Galli-Valerio, *Centralbl. f. Bakt.*, I. Orig., 1915, LXXVI, 516; Castellani, *Presse méd.*, 1917, XXV, 377; Thompson, *Brit. Med. Jour.*, 1918, II, 709; Violle, *Rev. gén. de clin. et de thérap.*, 1918, XXXII, 144; Nolf and Spehl, *Arch. Méd. Belges*, 1918, LXXI, 1; Farah, *Presse méd.*, 1919, XXVII, 774; Lewis U. S. *Naval Med. Bull.*, 1920, XIV, 140; Nolf, *Arch. Int. Med.*, 1920, XXV, 429; Vaccarezza, *Rev. de la Assoc. Méd. Argentina*, 1920, XXXII, 173; Trocello, *Ann. Med. Nov. e Colon*, 1920, XV, No. 2; Browne, *Jour. Trop. Med. & Hyg.*, 1920, XXIII, 225; Mason, *Bull. Johns Hopk. Hosp.*, 1920, XXI, 435; Tanon, *Médecine*, 1920, II, 220; Levy, *New York Med. Jour.*, 1921, CXIII, 186; Mendelson, *Jour. Trop. Med. & Hyg.*, 1921, XXIV, 59; Faill, *Tubercle*, 1921, II, 401; Bloedorn and Houghton, *Jour. Am. Med. Assoc.*, 1921, LXXVI, 1559; Segura and Puccio, *Semana Méd.*, 1921, XXVIII, 332.

CHAPTER II

ORAL, NASAL, AURAL, AND CONJUNCTIVAL SECRETIONS

I. ORAL SECRETIONS

(1) General Considerations.

The oral secretion is a mixture derived from the various buccal glands, the submaxillary, sublingual, parotid, and mucous glands. To this secretion has been given the name saliva. It is a colorless, odorless, and tasteless fluid, which appears somewhat stringy and frothy, separating on standing into two layers, the upper one of which is clear and the lower one cloudy. The function of this secretion is to moisten the mouth and throat and, also, to aid in swallowing the food as well as partially to digest the starchy food through the action of a specific ferment (ptyalin) which it contains. The normal daily amount of saliva secreted is usually about 1,500 c.c., this quantity varying under the influence of many factors, both physiologic and pathologic. The specific gravity ranges between 1.002 and 1.009 giving a total solid content of 3 to 12 grams. Its reaction is alkaline, corresponding to 0.006 to 0.48 per cent. of sodium hydrate.¹ While the reaction of the saliva is normally always alkaline, we occasionally find an acid reaction, especially in children and in the early morning hours, due to the production of lactic acid by the bacteria which are always present in the mouth. Likewise we find an acid reaction especially in conditions associated with acidosis, such states very frequently leading to dental caries and to many other irritative conditions of the mouth. The work of Talbot along this line is especially interesting.²

The chemical composition of the saliva does not have any great clinical significance³ with the exception of the presence of the sulphocyanates, the nitrites, and the characteristic ferment ptyalin. These substances seem to have some importance both from a diagnostic and symptomatic standpoint, so that a few remarks may be timely. The presence of potassium sulphocyanate (KCNS) is more or less characteristic of normal saliva and may be detected as follows: Collect a few c.c. of saliva before meals and allow this to filter. Add a few drops of hydrochloric acid and then a drop or two of ferric chlorid solution, when a distinct red color will be observed, whose depth will depend upon the amount of sulphocyanate present. It has been stated that heat should be applied in this test, but the writer has never found it necessary as the characteristic reaction almost invariably appears in the cold. This color disappears on the addition of mercuric chlorid solution, which fact may serve to differentiate it from the similar one given by the saliva of opium habitues

¹ Michaelis and Pechstein (*Biochem. Ztschr.* 1914, LIX, 77) have found the P^H value of saliva to be 6.9, that is, almost neutral from the physico-chemical standpoint. See also, Bloomfield and Huck, *Bull. Johns Hopk. Hosp.*, 1920, XXXI, 118.

² See Lafarga, *Rev. de la Asoc. Méd. Argentina*, 1921, XXXIV, 1152.

³ See Herzfeld and Stocker (*Zentralbl. f. inn. Med.*, 1913, XXXIV, 753) for a discussion of uric acid in saliva. Rathery and Binet (*Presse méd.*, 1920, XXVIII, 263) have shown that the salivary glands are involved in the diabetic process and that glucose may be eliminated in the saliva as well as or in place of its elimination in the urine.

PLATE III.



LEPTOTHRIX AND SPIROCHETA BUCCALIS. (UNSTAINED SPECIMEN.)

and due to meconic acid. Very little pathologic significance has been attached to variations in the amount of the sulphocyanate in the saliva, but it is interesting to note that in many cases of diabetes as well as in cases of severe stomatitis this substance is frequently absent. In pellagra the sulphocyanate content shows a rather marked decrease.¹

The nitrites may be detected by the more delicate tests used in water analysis, as their amount is usually not sufficient for the ordinary qualitative tests. A very good test is the use of the Griess-Ilosvay reagent ($\frac{1}{2}$ gram of sulphanilic acid is dissolved in 150 c.c. of dilute acetic acid and treated with $\frac{1}{10}$ gram of naphthylamin dissolved in 20 c.c. of boiling water. On standing, a blue sediment forms which is separated and dissolved in 150 c.c. of dilute acetic acid). On treating 10 c.c. of saliva with a few drops of this reagent and heating, a red color will develop in the presence of nitrites.

The most important constituent of saliva is the ptyalin which has a definite hydrolytic action upon starch, converting this polysaccharid into maltose through the intermediate stages of erythrodextrin² and achroödextrin. This action may be readily seen by treating a little starch paste with a few c.c. of filtered saliva and placing the vessel in the incubator for 10 to 15 minutes. At the end of this time iodine solution is added when a distinct red color or an entire absence of color will be noticed. It is to be remembered here that starch, treated with iodine, is colored blue. Careful work by Litmanowicz³ has shown that the diastatic power of saliva is unaffected by physiologic or pathologic variations in general body functions.⁴

(2) Microscopic Examination.

On allowing saliva to stand it separates into two distinct layers, the upper one clear and containing the liquid portion, while the lower is cloudy and contains the morphological elements. In the microscopic examination of this lower layer we observe many epithelial cells in the form of large, irregular, squamous cells which are derived from the mucous membrane of the mouth and tongue. The number of these cells present depends, of course, upon the erosion to which the mouth has been subjected by various irritants either of the food or of disease. The characteristic cells of the saliva are the salivary corpuscles, which resemble the leucocytes, but are larger and more granular. Occasionally red blood-cells are seen, but these have no direct significance other than to denote ulcerative or markedly irritative conditions somewhere in the nasopharynx. Beside these constituents of the saliva, we find, microscopically, many micro-organisms of the mold, yeast, and bacterial types. The bacteria are always present in the mouth as they are taken in with the air, food, and drink. Few of these have any direct significance, although the *spirochæta buccalis* and microdentium should be borne in mind, especially

¹ See Sullivan and Jones, Pub. Health Reports, U.S.P.H., 1919, XXXIV, 1068; Sullivan and Dawson, Jour. Biol. Chem., 1921, XLV, 473.

² See Blake, Jour. Am. Chem. Soc. 1920, XLII, 2673.

³ Zentrabl. f. d. ges. Physiol. u. Path. des Stoffw., 1909, IV, 81.

⁴ See Hirata, Biochem. Ztschr., 1912, XLVII, 167; van Haefl, Nederl. Tijdschr. v. Geneesk., 1915, LIX, 307; Lubimoff, Russk. Vrach., 1915, XIV, 269. Biedermann, Fermentforsch., 1916, I, 385; Rockwood, Jour. Am. Chem. Soc., 1919, XLI, 228; McGuigan, Jour. Biol. Chem., 1919, XXXIX, 273; Grimbert, Jour. Pharm. Chem., 1919, XIX, 244.

when an examination is being made for the *spirochaeta pallida*.¹ The former is differentiated from the latter by the fact that its ends lie upon a line drawn longitudinally through the center of its spirals, while such a line drawn through the *pallida* lies above and below its ends. Moreover, it should be remembered that the smegma bacillus is an occasional habitant of the mouth and throat and may occur in specimens of sputum, giving rise to the assumption of the presence of tubercle bacilli unless proper means of identification are used. Simon has pointed out an interesting fact that the majority of the micro-organisms which are constantly present in the mouth cannot be cultivated on artificial media, while the temporary invaders easily develop. Many pathogenic bacteria have been found in the mouth of the healthy subject.² This is interesting clinically as showing the constant danger to which we are all subject, in case our resistance becomes lowered. The writer recalls that the most virulent culture of pneumococci obtained from 200 throats, both diseased and normal, was from his own at the time when he was in perfect condition and showed no symptoms thereafter. Beside the pneumonia organism, streptococci, influenza and diphtheria bacilli are frequently found in the mouths of perfectly healthy individuals. Molds and yeast fungi are rarely found in the saliva during health, but they are present in pathological conditions.

(3) Pathologic Changes.

The normal daily secretion of the saliva is, as stated above, about 1,500 c.c. The composition of the secretions of the various glands, which contribute to the mixed secretion, differs rather widely, the one from the other. We may, therefore, have changes, not only in amount of saliva, but, also, in the quality, depending on the diseased condition of one or more of these glands. The quantity of saliva is diminished in inflammation of the salivary glands, such as in parotitis, in all febrile diseases, in diabetes, and in nephritis. The secretion is also diminished by the therapeutic use of preparations of belladonna and of opium. It is increased by certain poisons, such as pilocarpin and mercury, by excessive irritation with acids and alkalies, and, also, by irritations arising from carious teeth. Occasional cases have been reported of a greatly increased amount of saliva through some obscure nervous reflex, while such a condition is not unusual in pregnancy. An increased flow of saliva is known as salivation or *ptyalism*. In determining whether or not salivation really exists, observation will frequently show increased amounts of saliva at all times. In some cases, however, it is necessary to measure the amount and, also, to make later chemical examinations of the saliva. The best way of obtaining saliva free from contamination is to wash the mouth thoroughly

¹ See Thibaudeau, Jour. Am. Med. Assn., 1912, LIX, 446; Hoffmann, Deutsch. med. Wchnschr., 1920, XLVI, 257.

² See Küster, Kolle and Wassermann's Handbuch, 1913, VI, 435; Moorehead, Jour. A. M. A., 1916, LXVII, 845; Billings, Ibid., 847; Irons, Ibid., 851; Van Dyke, Jour. Am. Med. Assoc., 1920, LXXIV, 448; Olitsky and Gates, Ibid., 1497; Davis, Ibid., LXXV, 702; Bloomfield, Am. Rev. Tuberc., 1920, IV, 247; Jour. Am. Med. Assoc., 1921, LXXVII, 187; Bull. Johns Hopk. Hosp., 1921, XXXII, 33 and 121; Davis, Jour. Inf. Dis., 1921, XXIX, 524; Olitsky and Gates, Jour. Exp. Med. 1921, XXXIII, 125, 361, 373, 713; Ibid., XXXIV, 1; Ibid., 1922, XXXV, 553; Caylor and Dick, Jour. Am. Med. Assoc., 1922, LXXXVIII, 570.

with a solution of sodium bicarbonate, brush the teeth thoroughly with the same solution, and then rinse out the mouth with cold water. On now touching the inner surface of the teeth or the edge of the tongue with a glass rod that has been dipped into dilute acid, saliva will be seen pouring into the mouth from many points. This saliva is then collected in clean receptacles and the quantity measured.

Variations in the reaction of the saliva are not uncommon in pathologic conditions. In various intestinal diseases with which we may have an associated stomatitis, an acid reaction is frequently noted. Also in fevers, diabetes, starvation, and other conditions giving rise to acidosis (overloading of the system with acid products) the reaction of the saliva is always acid. Strauss and Cohn believe that the saliva is alkaline, even under pathological conditions.

Coating of the Tongue.

A coating of the tongue is practically always abnormal, as the normal appearance is a bright reddish color with no visible deposits.¹ A change in the normal appearance of the tongue has so long been indicative, in the minds of the profession, of disturbed conditions not only in the mouth, but in the stomach and the bowels, that one should always take into consideration any such change. In severe infectious fevers a brownish coating with a furred appearance is practically always seen. This consists of remnants of food and of incrustated blood, along with large numbers of micro-organisms² and dark desquamated epithelial cells. The white coating contains no blood and is more indicative of simple gastro-intestinal disturbance than is the brown coating. The so-called "tartar" which forms upon the teeth seems to consist of deposited calcium carbonate and contains many actively motile spirochete as well as large segmented leptothrices, along with leucocytes and epithelial cells.

Pharyngomycosis Leptothrica.

In many pathological conditions of the throat, such as tonsillitis, diphtheria, and thrush, we frequently find the tonsillar and other buccal structures covered with a coating which is, in many cases, a distinct membrane containing the pathogenic organisms in large numbers. Many perfectly normal subjects complain of the formation, in the tonsillar crypts, of plugs of material which are easily removed by pressure. These are frequently found in patients subject to tonsillitis, but, also in those showing no pathological conditions of the tonsils and are closely related to Dittrich's plugs, which have been discussed.

In the pyoid masses of pharyngomycosis leptothrica, one finds large numbers of lymphocytes, epithelial cells and long segmented fungi, the leptothrices buccalis, which are colored bluish-red by a solution of iodopotassic iodid. In such conditions the polynuclear neutrophiles are present in only

¹ See Schilling, *Deutsch. Arch. f. klin. Med.*, 1914, CXIII, 622.

² Catacuzene (*C. R. soc. biol.*, Paris, 1914, LXXVII, 449 and 452) describes a pleomorphic organism in the scrapings of the tongue in scarlet fever. In smears stained by Giesma stain several forms are seen, minute ovoid or elongated bodies stained an intense blue and presenting at the center or near one pole a chromatic spot.

small numbers. In some cases patches of these fungi extend over quite an area of the tonsils so that the appearance may be one of the formation of a diphtheritic membrane, although microscopic examination will at once clear up the diagnosis.¹

Diphtheria.

One of the most important examinations of the oral cavities consists in the detection of the diphtheria bacillus (Klebs-Löffler bacillus), as an early diagnosis of this disease frequently enables the physician to institute antitoxin treatment. Such an examination should never be omitted in any case of suspected sore throat, especially where any membranous patches are present.² By means of a stout platinum loop or a swab of cotton a piece of membrane or a portion of the exudate is scraped from the throat. This material is then spread over the surface of Löffler's blood serum and is allowed to incubate at 37°C. for six to eight hours. This period of incubation is of some importance as it has been definitely shown that at the end of six to eight hours the diphtheria organism is the only one which will attract much attention, while if left for a longer time, other organisms, especially the streptococcus and staphylococcus, will so far outgrow the diphtheria bacillus that this latter may be unrecognizable unless the incubation be carried 36 hours, when the diphtheria bacillus then assumes the ascendancy. From this culture, cover-glass preparations are then made and stained for one to five minutes in Löffler's alkaline methylene blue solution. They are then rinsed in water, dried, and examined with the immersion lens.

Neisser's Stain.

This stain is supposed to differentiate the diphtheria bacillus from all others. The smear is stained for five minutes with a methylene blue solution (methylene blue, Grüber, 1 gram, 20 c.c. of 96 per cent. alcohol, glacial acetic acid 50 c.c., and water 950 c.c.). The stain should be filtered before use. The specimen is heated gently during the staining process and the dye renewed as the stain evaporates. Wash in water³ and stain for two minutes with an aqueous solution of Bismarck brown or, better, a dilute solution of safranin. The polar bodies will be stained a deep blue, while the body of the bacillus will take a light brown or red color.⁴

Microscopically, the stained organism appears as a slightly curved rod, but especially characteristic are the bizarre forms, such as rods with alternate staining and nonstaining portions, rods with distinct deeply staining polar bodies, club-shaped or "narrow-waisted" rods, many of which lie together in distinctly parallel lines.

¹ Conlon (Jour. Am. Med. Assn., 1913, LX, 900) has reported the *aspergillus niger* as a causative factor in infection of the pharynx. Basile (Policlinico, 1917, XXIV, 88 and 97) reports a study of pharyngeal blastomycosis.

² See Neisser and Gins, *Kolle und Wassermann's Hand. d. path. Mikroorg.*, 1912, V, 931.

³ Gins (Deutsch. med. Wchnschr., 1913, XXXIX, 502) advises a short (2 to 3 seconds) treatment at this point with Lugol's solution containing 1 per cent. of lactic acid. See, also, Tamura, *Ztschr. f. physiol. Chem.*, 1914, LXXIX, 289; Neilsen, *Hospitaltid.*, 1915, LVIII, 225.

⁴ See Raskin, *Deutsch. med. Wchnschr.*, 1911, XXXVII, 2384; also, Ponder, *Lancet*, 1912, CLXXXIII, 22; Kinyoun, *Am. Jour. Pub. Health*, 1915, V, 246; Mood, *Southern Med. Jour.*, 1915, VIII, 482; Nicholls, *Jour. Lab. & Clin. Med.*, 1921, VII, 180.

PLATE IV.



Katharine Hill.

DIPHTHERIA BACILLI SHOWING POLAR STAINING. (NEISSER METHOD, COUNTER STAINED WITH SAFRANIN.)

Albert's Stain.

This stain¹ appears to possess advantages over others advocated so that the author recommends it for routine use. Two solutions are employed:

SOLUTION I

Toluidin blue.....	0.15 gram
Methyl green.....	0.20 gram
Acetic acid (glacial).....	1.00 c.c.
Alcohol (95 per cent.).....	2.00 c.c.
Water (distilled).....	100.00 c.c.

After standing for one day, the solution is filtered and is ready for use.

SOLUTION II

Iodin.....	2 grams
Potassium iodid.....	3 grams
Water (distilled).....	300 c.c.

This solution is ready for use as soon as the iodine is entirely dissolved.

Smears are made on slides or cover glasses, fixed by heat, stained with Solution I for one minute, washed with water, dried with good absorbent filter paper, stained with Solution II for one minute, washed with water and dried with filter paper, and examined with the immersion lens. The granules of the diphtheria bacilli are stained black and stand out in marked contrast to other elements in the microscopic field; the bars of the bacilli take a dark green and the intermediate portions a light green color. Other bacteria are, also, stained a light green.

Diphtheria bacilli may be found in the throat for weeks after all symptoms have disappeared² so that it is wise to enforce isolation of the patient until three consecutive negative cultures, taken at intervals of from one to three days from both nose and throat, are obtained. In causing the disappearance of these bacilli from the throats of "carriers" the method of spraying the nose and throat every three hours with a 24-hour bouillon culture of *staphylococcus aureus*, as advanced by Schiötz (Ugesk. f. Laeger, 1909, LXXVI, 1373), has proven very satisfactory. However, Bissell (Jour. Am. Med. Assn., 1913, LXI, 1393) believes the method is questionable as it leads to false security owing to the fact that the few diphtheria bacilli actually left in the deeper structures are masked by the numerous organisms of the sprayed culture.³ If the carriage of the organisms persists, tests of virulence should be made.

¹ Jour. Am. Med. Assoc., 1921, LXXVI, 240.

² See Markl and Pollak, Wien. klin. Wchnschr. 1913, XXVI, 1617.

³ See Womer, Jour. Am. Med. Assn., 1913, LXI, 2293. Hektoen and Rappaport (Jour. Am. Med. Assn., 1915, LXIV, 1985) advise the use of kaolin, either swallowed or given as an insufflation, for this purpose. See, also, Perkins, Miller and Ruh, Jour. Infect. Dis., 1916, XVIII, 607; Kolmer and Moshage, Ibid., XIX, 1; Geiger, Kelly and Bathgate, Jour. A. M. A., 1916, LXVI, 645; Ott and Roy, Ibid., 800; Friedberg, Ibid., 810; Goff, Ibid., 941; Ruh, Miller and Perkins, Ibid., 941; Rappaport, Ibid., 943; Lewis, Ibid., 1535; Rabinoff, Ibid., LXVII, 1722; Kolmer, Woody and Moshage, Am. Jour. Dis. Child., 1916, XI, 257; Walthall, Ibid., XII, 140; Weaver, Jour. Infect. Dis., 1917, XX, 125. Arloing, Medicine, 1910, I, 140; Stevenin, Ibid., 168; Gloyne, Jour. Am. Med. Assoc., 1920, LXXIV, 83; Huet, Nederl,

Occasionally in examination of smears from the throat, true diphtheria bacilli may be confounded with pseudo-diphtheria bacilli,¹ and in examination of other specimens, such as those taken from the eye, the bacillus xerosis may be confusing. These different organisms are best differentiated by the study of their action in fermenting or not fermenting certain sugars. According to Knapp, the pseudo-bacilli will ferment none of the sugars, the diphtheria bacilli will ferment dextrose, mannite, maltose, and dextrin, but not saccharose, while the xerosis bacillus ferments dextrose, mannite, maltose and saccharose (cane sugar), while it does not ferment dextrin. (See Chapter XI.)

Vincent's Angina (Ulceromembranous Angina and Stomatitis).

In this condition, smears taken from the throat, as well as the free saliva will be found to contain many organisms of two special types, the first, spirilla,

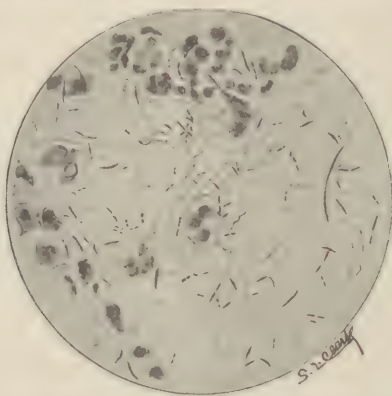


FIG. 12.—Vincent's spirillum and bacillus. (Coplin.)

and the second, long fusiform bacilli. Usually both of these types are found together, but occasionally the spirilla are absent. The spirilla usually measure from 36 to 40 microns in length and $\frac{1}{2}$ micron in breadth, while the bacilli are 6 to 12 microns in length and are somewhat thicker in the center than at the end. These organisms may be stained with Löffler's methylene blue, gentian violet, or dilute carbol-fuchsin, but they decolorize with Gram's method.²

Tijdschr. v. Geneesk., 1920, I, 303; Brownelle, Lancet, 1920, I, 706; Wadsworth, Jour. Am. Med. Assoc., 1920, LXXIV, 1633; Henry, Ibid., LXXV, 1715; Guthrie, Gelien and Moss, Bull. Johns. Hopk. Hosp., 1920, XXXI, 388; Ibid., 1921, XXXII, 100; Weaver, Jour. Am. Med. Assoc., 1921, LXXVI, 831; Dujarric de la Rivière, Bull. Acad. de Méd., 1921, LXXXV, 542; Kritzler, Ztschr. f. Geburtsh. u. Gynäk., 1921, LXXXIV, 170; Schoedel, Jahrb. f. Kinderhke., 1921, XCVI, 273; Spitzner, Ibid., 270; Gray and Meyer, Jour. Inf. Dis., 1921, XXVIII, 323; Simmons, Wearn & Williams, Ibid., 327; Marshall and Guthrie, Bull. Johns. Hopk. Hosp., 1922, XXXIII, 110.

¹ See Meader, Jour. Infect. Dis., 1919, XXIV, 145. Perkins and Spreng (Jour. Inf. Dis., 1921, XXIX, 513) call attention to the occasional presence in throat cultures of organisms of the *B. lactimorbi* group, which closely simulate *B. diphtheria*.

² The organism has been recently cultivated, but inoculation experiments have been unsuccessful. See Gins, Kolle and Wassermann's Handb. d. path. Mikroorg., 1912, V, 1003. See McClintock, Am. Jour. Med. Sc., 1917, CLIII, 250; Campbell and Dyas, Jour., A. M. A., 1917, LXVIII, 1596. This organism is more properly speaking, to be classed among the spirochetes. See Gifford, Jour. Bacteriol., 1920, V, 305; Reckord and Baker, Jour. Am. Med. Assoc., 1920, LXXV, 1620.

Streptococcic Sore Throat.

During recent years several milk-borne epidemics of sore throat with severe constitutional symptoms have occurred, especially in Boston, Chicago and Baltimore, of which the causative organism is a peculiar streptococcus (the *streptococcus epidemicus*). In smears from the throat and tonsillar exudates this highly virulent organism occurs in short chains, the spherical cocci appearing in twos in the chain. They are strongly Gram-positive and are surrounded by a definite capsule. They produce a relatively narrow zone of hemolysis on blood agar but little or no greenish color.¹

Gonorrheal Stomatitis.

In this condition the usual changes of infection are observed along with the appearance of the gonococci in the smears. Boston reports several cases of supposed gonorrheal stomatitis in which cultural methods showed the



FIG. 13.—*Oidium albicans*. (Kolle and Wassermann.)

absence of this organism, although the smears showed the presence of intracellular Gram-negative diplococci. Such reports are not surprising in view of the fact that so many saprophytic diplococci (such as the *diplococcus flavus* and *crassus*) are found which may or may not stain by Gram's method. The almost constant presence of the *micrococcus catarrhalis* must be borne in mind.

Thrush.

This is a condition most commonly seen in children, but may occur in adults, especially in those with tubercular tendencies. The saliva in this con-

¹ See Davis and Rosenow, Jour. Am. Med. Assn., 1912, LVIII, 773; Hamburger, Ibid., p. 1109; Davis, Ibid., pp. 1283 and 1852; Capps and Miller, Ibid., p. 1848; Heinemann, Ibid., 1912, LIX, 716; Leutscher, Ibid., p. 809; Frost, Pub. Health Reports, 1912, XXVII, 1889; Stokes and Hachtel, Ibid., p. 1923; Winslow, Jour. Infect. Dis., 1912, X, 73; Hamburger; Bull. Johns Hopkins Hosp., 1913, XXIV, 1; Capps, Jour. Am. Med. Assn., 1913, LXI, 723; Mann, Jour. Infect. Dis., 1913, XII, 481; North, White and Avery, Ibid., 1914, XIV, 124; Capps and Davis, Jour. Infect. Dis., 1914, XV, 130; Arch. Int. Med., 1914, XIV, 650. Rosenow and Moon, Jour. Infect. Dis., 1915, XVII, 69; Kirkland (Brit. Med. Jour., 1914, I, 419) reports a case from Cheltenham which belongs in this group; Bray, Jour. Am. Med. Assn., 1915, LXIV, 1127; Krumwiede and Valentine, Jour. Med. Research, 1915, XXXIII, 231; Winslow and Hubbard, Jour. Infect. Dis., 1916, XVIII, 106; Rosenow and Hess, Jour. A. M. A., 1917, LXVIII, 1305; Henika and Thompson, Ibid., 1307; Davis, Jour. Infect. Dis., 1918, XXIII, 550; Howard and Orcutt, Jour. Exper. Med., 1920, XXXI, 49. Sharp, Norton and Gordon (Jour. Inf. Dis., 1922, XXX, 372) report an epidemic of sore throat in which a hemolytic streptococcus (belonging to Holman's group *S. infrequens*) and a type 4 pneumococcus were associated.

dition is usually acid and somewhat increased in amount. Microscopic examination of the membrane shows many epithelial cells, leucocytes, and much granular detritus with a network of branching band-like formations, showing distinct segments.¹ The contents of the segments are clear and usually contain two highly refractive granules, one at each pole. This organism is known as the *Oidium albicans*. It stains well with the ordinary aqueous methylene blue solution.

Oral Endamebiasis.

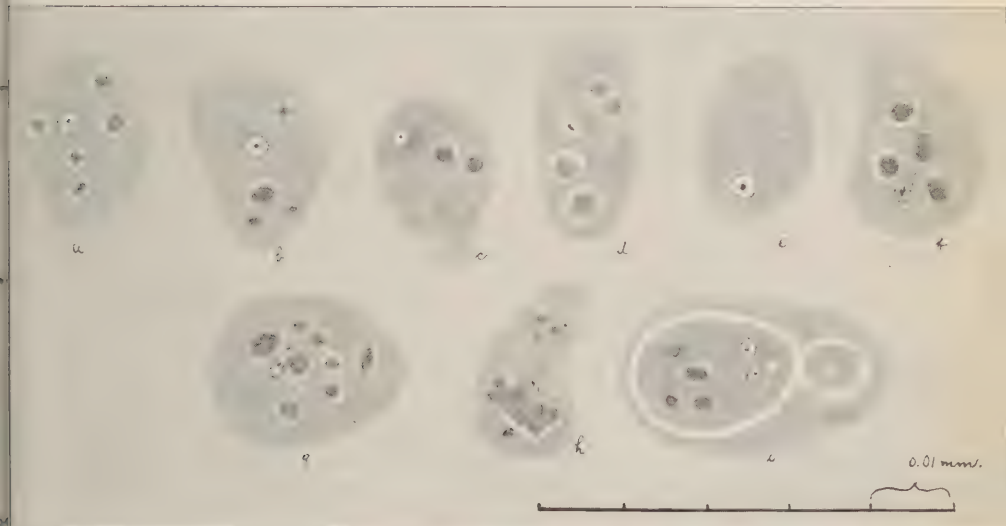
The condition, known as pyorrhea alveolaris or Riggs' disease, has for years been regarded as a form of bacterial infection in which local irritative processes and systemic disturbances are looked upon as predisposing factors. Many types of bacteria, such as streptococci, pneumococci and staphylococci, have been found and isolated from the pus pockets about the roots of the teeth, but these cannot be regarded as the only etiologic factors in this condition, at least in the majority of cases.²

While it has been known for years that parasites, especially those of the ameba group, were very frequently found in the mouth and in the soft tartar about the teeth, these were regarded as innocuous invaders, except in a few isolated cases. During recent years a great amount of work, following the first communication of Barrett, has centered about these amebæ and their association with the lesions of pyorrhea, until at present their pathogenic importance is being freely discussed. There is little question, according to Smith and Barrett, that we must now "regard these parasites as either directly causative of a large class of gingival and alveolar pyorrheas or as important members of a symbiotic chain with one or other of the numerous associated vegetable micro-organisms, in the production of these lesions." It is to be said that the proof of the pathogenic importance of the endamebæ in Riggs' disease rests upon their almost constant presence in the suppurating pockets of pyorrhea (while they are rarely found in healthy mouths), and the prompt removal of both suppuration and of the endamebæ when emetin (first advocated by Roger) is administered locally or generally. The organisms have not yet been cultivated, so that the question of conformity with the classic postulates of Koch has not been investigated. Further, many of the cases supposedly cured by emetin show recurrences after a few weeks' freedom from treatment. While there is no question of the presence of these amebæ in cases of pyorrhea,

¹ Ashford (Jour. Am. Med. Assn., 1915, LXIV, 810 and 1893) reports the finding, in cases of sprue, of a yeast (*monilia psilosis*) not hitherto described. See Ashford, Am. Jour. Med. Sc., 1915, CL, 680; 1916, CLI, 520 and 1917, CLIV, 157; Michel, *Ibid.*, 177, and Wood, *Ibid.*, 1915, CL, 692, and Jour. A. M. A., 1919, LXXIII, 165; Oliver, Jour. Am. Med. Assoc., 1920, LXXIV, 27; Castellani, Jour. Trop. Med. and Hyg., 1920, XXIII, 17; Boyd, Southern Med. Jour., 1920, XIII, 229; Heaton, Indian Jour. Med. Res., 1920, VII, 810; Steinert, Ztschr. f. Kinderhkde., 1920, XXV, 83; Magalhães, Brazil-Médico, 1920, XXXIV, 431; Wood, Jour. Trop. Med. & Hyg., 1920, XXIII, 201; del Valle Atilas, Bull. Porto Rico Med. Assoc., 1920, XIV, 103; Fineman, Jour. Inf. Dis., 1921, XXVIII, 185; Krauss, Am. Jour. Trop. Med., 1921, I, 119; Hannibal and Boyd, *Ibid.*, 165; Thomas, Jahrb. f. Kinderhkde., 1921, XCVI, 95; Bovaird, Jour. Am. Med. Assoc., 1921, LXXVII, 753.

² See Nodine, Dental Cosmos, 1914, LVI, 969; Gilmer and Moody, Jour. Am. Med. Assn., 1914, LXIII, 2023; Billings, *Ibid.*, 2024; Mayo, *Ibid.*, 2025; Rosenow, *Ibid.*, 2026; Craig, *Ibid.*, 2027; Hartzell and Henrici, *Ibid.*, 1915, LXIV, 1055; Hoxie, Jour. A. M. A., 1915, LXV, 1908; Lescossier, *Ibid.*, 1917, LXVIII, 414; Medalia, *Ibid.*, 798.

PLATE V.



CAMERA LUCIDA DRAWINGS OF *ENDAMEBA GINGIVALIS* (GROS), STAINED WITH IRON HEMATOXYLIN.
 (A. J. Smith and M. T. Barrett, *Jnl. of Parasitology*.)

it has not been firmly established, as yet, that they are truly pathogenic rather than merely "members of the symbiotic chain."

Several forms of endamebæ have been described as habitants of the buccal and dental tissues, some of which were supposed to be pathogenic. These various amebæ are more or less similar in their general characteristics, so that it is more than probable that the one, now first recognized by Barrett and a little later by Bass and Johns as almost constantly associated with pyorrhea, is the same as was long ago described by Gros, Steinberg, Grassi, Flexner and Prowazek. It is no more than just, therefore, that this ameba, whose pathogenicity is being investigated, should be styled *Endameba*¹ *gingivalis* (Gros) and not *Entameba buccalis* (Prowazek), as some call it.

These endamebæ, as studied by Smith and Barrett, show the following characteristics: "Naked parasitic amebæ of usual diameter in resting examples of 0.030 to 0.035 mm. (with exceptional instances reaching 0.040 or slightly above); with refractile and faintly greenish-tinted hyaline ectosarc well defined from the granular endosarc, but sometimes so thin as to be easily overlooked; endosarc granular, colorless and in all but the more minute examples containing few of many digestion vacuoles in which globular detritus of leucocytic nuclei and red blood cells are commonly found along with bacteria; with a small (0.002 to 0.005 mm.) rounded nucleus, invisible or at best uncertainly distinguishable in the unstained specimen, usually central or sub-central in position, but at times eccentric (when the ingesta push it to one side). The nucleus is very poor in chromatic substance, vesicular, with a small "binnenkörper," sometimes showing a minute centriole, a clear space between it and the nuclear border containing no chromatin or at most a very few incomplete threads; and the border is represented by a thin but somewhat irregular line of chromatin, about which the writers are unable to recognize a further membrane. The degree of motility manifested by the parasite is fairly comparable to that of the dysenteric endameba. The pseudopods as a rule are few (one, two or three), usually broadly lobose and commonly attaining a maximum length of the diameter of the endosarc. The pseudopods are composed practically entirely of the ectosarc, the granular endosarc terminating at the base or extending but a little into the larger ones."

The material for examination is best obtained by scraping the bottom of the lesion in the peridental membrane between the tooth and alveolar process, as the endamebæ do not live on the normal gum margin but at the edge of the tissue which they are invading. As the secondary infection with pyogenic organisms causes a profuse flow of pus which distends the pocket, very few endamebæ can reproduce and live in this necrotic material. For this reason it is absolutely essential for success in this examination that most of this purulent material be removed from the old lesions and the *specimen for examination be obtained from the bottom of the lesions*. By means of a toothpick or, preferably, a Younger scaler No. 8 or 9, remove some of the material from the bottom of the lesions. If the specimen is to be examined in the fresh condition, mix the material with a drop or two of slightly warm normal saline

¹ The name endameba was first suggested by Leidy in 1879 and should take precedence of the term entameba advocated by Casigrandi and Barbagallo in 1897.

solution on a warm slide and place over this a cover-glass. Examine at once with the high-power dry lens. Careful study will reveal the motile organisms showing the characteristics mentioned above. If the organisms are not actively motile, it is not the simplest matter to recognize them in the fresh state.

For general work the stained specimen is, probably, more satisfactory for examination, as differentiation is more certain. Obtain the material as above mentioned and scrape it onto a clean slide with a toothpick, spreading it out into a thin layer with the flat side of the toothpick. Care should be taken not to rub the material back and forth as this may break up the amebæ. Allow the slide to dry in the air and fix by passing once or twice through the flame. Cover the smear with carbol-fuchsin for 10 to 15 seconds, wash off the excess of stain with water and stain with Löffler's methylene blue for 30 seconds.¹ Wash in water, dry and examine with the immersion lens. Properly prepared specimens should have a deep purple color. The red blood cells stain a deep red. Pus cells show a bright purple nucleus with a light pink protoplasm. Tissue cells are larger than the pus cells and appear reddish with a small purple nucleus. Bacteria may be stained either red or blue, depending on their type. The endamebæ vary in size from cells somewhat smaller than a pus cell to those several times as large. The endoplasm stains a deep blue, while the irregular border of ectoplasm stains purple. A small round or oval centric nucleus is observed staining a deep port-wine color, while many inclusion bodies (contained in vacuoles) may be seen staining a deep purple. Usually the entire endameba appears to be surrounded by a clear zone, due to retraction during drying.²

II. NASAL SECRETION

This secretion seems to be of pathologic significance only in infectious conditions. Normally, the nasal secretion is comparatively scanty, clear.

¹ Dupray (Jour. A. M. A., 1915, LXVI, 507) recommends the use of a carbolized thionin solution.

² Gros, Bull. Soc. imp. nat. de Moscow, 1849, XXII, 549; Steinberg, Souremenaya meditsina, Dissert. Kiev, 1862; Grassi, Gazz. med. Ital.-Lomb., 1879, XXXIX, 446; Flexner, Bull. Johns Hopkins Hosp., 1892, III, 104; Kartulis, Ztschr. f. Hyg., 1893, XIII, 9; Prowazek, Arbeiten a. d. Kais. Gesundheitsamte, 1904, XXI, 42; Leyden and Löwenthal, Charité Ann., 1905, XXIX, 3; Verdun and Bruyant, L'Echo méd., du Nord, 1907, XI, 375; Bruyant and Pelissier, Ibid., 1909, XIII, 301; Brumpt, Précis de Parasitol., Paris, 1910; Barrett, Dental Cosmos, 1914, LVI, 948 and 1345; Chiavaro, Editorial in Dental Cosmos, 1914, LVI, 1089; Austral. Jour. Dent., 1914, XVIII, 343; Dental Review, 1914, XXVIII, 1122; Bass and Johns, New Orleans Med. and Surg. Jour., 1914, LXVII, 456 and 671; Smith, Middleton and Barrett, Jour. Am. Med. Assn., 1914, LXIII, 1746; Evans and Middleton, Ibid., 1915, LXIV, 422; Bass and Johns, Ibid., 533; Howe, Dental Cosmos, 1915, LVII, 307; Brit. Dental Jour., 1915, XXXVI, 464; Talbot, Dental Cosmos, 1915, LVII, 485; Jour. Am. Med. Assn., 1915, LXIV, 928; Rogers, Indian med. Gaz., 1915, I, 121; Stewart, Med. Record, 1915, LXXXVII, 798; Dental Cosmos, 1915, LVII, 605; Sturridge, Ibid., 780; Johns, Interstate Med. Jour., 1915, XXII, 529; Smith, Jour. Am. Med. Assn., 1915, LXIV, 1567; Smith and Barrett, Jour. Parasitol., 1915, I, 159; Brunelles and Ginsberg, New York Med. Jour., 1915, CII, 554; Williams, von Shelly and Rosenberg, Proc. New York Path. Soc., 1915, XV, 334; Bass and Johns, Alveodental Pyorrhea, Saunders, Phila., 1915; Hecker, Am. Jour. Med. Sc., 1915, CXLIX, 889; Smith and Barrett, Jour. Parasitol., 1915, II, 54; Lynch, Jour. A. M. A., 1915, LXV, 2077; Johns, Amer. Jour. Trop. Dis. and Prev. Med., 1916, III, 307; Sanford, Surg., Gyn., and Obs., 1916, XXII, 27; Price, Ibid., 37; Craig, Jour. Infect. Dis., 1916, XVIII, 220; Moody, Ibid., 1916, XIX, 515; Hecker, Ibid., 729; Mitchell, Culpepper and Ayer, Jour. Med. Research 1916, XXXV, 51; Evans, Middleton and Smith, Am. Jour. Med. Sc., 1916, CLI, 210; Nowlin, Jour. Parasitol., 1916, III, 143 and 1917, IV, 21.

tenacious, odorless, salty in taste, and alkaline in reaction. It is largely composed of mucus, showing squamous and ciliated epithelium in abundance,¹ occasionally leucocytes, large numbers of bacteria and Charcot-Leyden and triple phosphate crystals. The bacterial content is made up of both pathogenic and non-pathogenic organisms.

Pathologic Changes.

In most acute infections the nasal secretion is at first diminished in amount, but soon becomes very profuse. This secretion shows the same appearance as does the normal fluid, but as ulceration ensues may be heavily loaded with pus-cells and bacteria. The chronic suppurative process in the nose may affect any or all of the accessory sinuses, so that we may have very severe conditions arising from simple ulceration. Frequently the ulcerative and membranous conditions spoken of above may extend from the mouth to the nose, so that distinct diphtheritic membranes are frequently found in the nasal cavities. Molds may develop to such an extent that true pathological lesions obtain.²

Rhinitis.

At present the infectious nature of the catarrhal conditions known as "colds" is generally accepted, many organisms, such as micrococcus catarrhalis, pneumococcus, streptococcus, influenza bacillus and others, having been associated in different cases with the etiology. It would seem that these organisms are, however, to be regarded as simply secondary invaders, in many cases at least, and become of importance only when the discharge is purulent.

Tunncliffe³ has found, in the mucoid stages of acute and chronic rhinitis, a delicate, curved, Gram-negative bacillus, which appears to have a direct pathogenic association with these conditions. While this *Bacillus rhinitis* of Tunncliffe is found in only 6 per cent. of normal noses, it was present in 98 per cent. of cases of acute rhinitis and in 90 per cent. of cases of chronic non-purulent rhinitis. It is, apparently, not found at all in purulent rhinitis, the other organisms mentioned above being the causative factors in this latter condition. There is little doubt that this organism has an etiologic relationship to acute and chronic rhinitis on account of its almost constant presence in the nose in such cases, its general absence from the normal nose, its ability to produce rhinitis experimentally with recovery in pure culture, and on account of the production, in cases of acute and chronic rhinitis and in persons injected with the bacillus, of specific opsonins and complement-binding bodies.

This organism may be found by spreading a fairly large amount of the nasal discharge (mucus) on a glass slide, drying and staining with carbol-fuchsin. Often a large number of microscopic fields may have to be examined before the organisms are found. They vary in length from 5 to 8 microns and from $\frac{1}{3}$ to $\frac{1}{2}$ micron in width. The ends are pointed or slightly rounded; generally the bodies are slightly curved, but may be straight, wavy or bent

¹ Proskauer (Berl. klin. Wchnschr., 1915, LI, 172 and 255) has shown that some of the cells may be melanotic.

² See Tilley, Jour. Laryngol., Rhinol. and Otol., 1915, XXX, 145.

³ Jour. Infect. Dis., 1913, XIII, 283; Ibid., 1915, XVI, 493. See Howell, Ibid., 456; also, Kruse, Munch. med. Wchnschr., 1914, LXI, 1547.

over at one end. A ring and an enlargement in the form of a ball are occasionally seen at one extremity. The organism is a strict anaerobe growing at 37°C. slowly, the colonies on slightly alkaline goat-blood agar appearing usually in about seven days as small, dull, round growths. The organism does not grow on plain agar. Specimens from cultures stain a little more deeply than those taken directly from the mucus, but they retain their Gram-negative characteristics.

Hay Fever.

In this condition the nasal secretion is found to be increased to a large extent at certain times of the day and much diminished at others, depending upon the paroxysms of the disease. Nothing of pathological importance has been found, however, in the examination of the nasal secretion in this condition, no specific organism having been identified.

Meningitis.

In some cases of meningitis the cerebrospinal fluid passes into the nasal cavity as a result of caries of the bones of the skull. This fluid may be distinguished by the fact that it contains practically no albumin, but does show the presence of a reducing substance which may or may not be sugar. This fluid may also contain the diplococcus intracellularis meningitidis of Weichselbaum. While this organism is not found in all cases of epidemic meningitis, yet it is found in many, so that the nasal secretion may be of some diagnostic importance from this standpoint.

In the course of glanders, leprosy, plague, pneumonia, typhoid fever, influenza, and many other infectious diseases, the characteristic organisms of these conditions may be found in the nasal secretion. Recently Goldberger and Anderson¹ have demonstrated the presence of the unknown virus of measles in the mixed buccal and nasal secretions.²

Occasionally concretions are found in the nose, but these rarely reach a large size and do not have very great pathologic significance. They are largely composed of vegetable fibers taken in by inhalation and cemented by mucus which is hardened by the deposition of lime salts.

In the condition known as ozena, the nasal secretion and fetid crusts, so characteristic of this disease, are found to contain numerous organisms of various types, among which one observes a peculiar cocco-bacillus which is difficult of cultivation and isolation. This organism was first noted by Perez, but it was not until the later studies of Hofer and his associates that it was practically established as the etiologic factor in this disease. To this micro-organism has been given the name *coccobacillus fœtidus ozenæ* (Perez).³

¹ Jour. Am. Med. Assn., 1911, LVII, 476 and 971; Am. Jour. Dis. Child., 1912, IV, 20.

² See also Lucas and Prizer, Jour. Med. Research, 1912, XXVI, 181.

³ See Hofer, Wien. klin. Wchnschr., 1913, XXVI, 1011; also, Abel and Hallwachs, Kolle and Wassermann's Handbuch, 1913, VI, 534; McGowan, Jour. Laryngol., Rhinol. and Otol., 1915, XXIX, 57; Guggenheim, Interstate Med. Jour., 1915, XXII, 129; Horn, Jour. Am. Med. Assn., 1915, LXV, 788; Horn and Victors, N. Y. Med. Jour., 1916, CIV, 1094; Ward, Jour. Infect. Dis., 1916, XIX, 153; Ward and Beaver, Jour. Lab. and Clin. Med., 1918, III, 348; Ferry and Noble, Jour. Bacteriol., 1918, III, 499.

III. THE AURAL SECRETION

Normally no secretions appear in the external ear, with the exception of that of cerumen, while the secretion of the middle ear and of the internal ear is normally inaccessible to examination. We find, therefore, that the chief importance which is attached to the clinical examination of the aural secretions, is entirely a pathological one. In catarrhal and inflammatory conditions of the external auditory canal, one finds naturally very large numbers of organisms, with which the disease may or may not be associated. In the chronic inflammatory processes of the middle ear, the more important organisms found are the pneumococcus, streptococcus pyogenes, staphylococcus pyogenes, bacillus pyocyaneus, the bacillus of Friedländer, the bacillus coli communis, the diplococcus intracellularis, the typhoid bacillus, and especially the diphtheria bacillus.¹ As disease of the middle ear is so commonly associated with disease of the naso-pharynx, it is possible to find in the discharge from the ear any organism which is causing trouble either in the nose or in the throat. Hamilton has shown the almost constant presence of the pseudo-diphtheria bacillus in the discharge of the running ears following scarlet fever.

It is not an uncommon thing to find certain inflammatory processes of parasitic origin in the external auditory canal. This condition is known as otomycosis² and is frequently caused by the aspergillus niger. Besides this organism, many other fungi belonging to this group have been found in the external ear. Among these we find the aspergillus flavus and fumigatus, the aspergillus nidum, the mucor septatus, the eurotium malignum, and the penicillium minimum. These parasites are very readily detected by removing a small portion of the mycotic mass and spreading it thinly on a slide. Add a small drop of water, apply a cover-glass, and examine under a high-power dry lens. For the details of structure of such organisms, bacteriological works should be consulted.

Besides these fungi we occasionally find in the external auditory canal larvæ of various insects. These larvæ may develop later into the full-grown insect and may be removed from the ear by the movements of the animal. In other cases such larvæ have incited inflammatory processes which are occasionally troublesome, as in the case reported by Richardson.

IV. THE CONJUNCTIVAL SECRETIONS

Under normal conditions the secretion of the conjunctiva and of the lacrimal gland concerns us only very little. It is in the course of an inflammatory process in which these secretions may be greatly increased and greatly changed by the inflammation that any clinical importance attaches to them. In inflammatory conditions of the conjunctiva we find certain organisms which

¹ Van Horn (Jour. Am. Med. Assoc., 1921, LXXVI, 32) reports a case of primary diphtheria of the middle ear. See, also, Blanchard, Ibid., 1922, LXXVIII, 1458. Cheatle (Jour. Laryngol., Rhinology and Otolaryngology, 1920, XXV, 6) describes two cases of Vincent's angina of external auditory meatus.

² See Cheatle, Jour. Laryngol., Rhinology & Otolaryngology, 1920, XXXV, 33; Koenig, Med. Record, 1920, XCVII, 956.

require identification in order that proper treatment may be instituted and the proper prognosis given.¹ It is to be recalled that the pseudo-diphtheria bacillus is practically always found in smears made from the conjunctival secretion, yet it is rarely, if ever, pathogenic in this situation.²

Pathologic Changes.

Diphtheritic Conjunctivitis.

In cases of conjunctivitis which are traceable to infection with the diphtheria organism, we frequently find the formation of an extensive membrane which consists of epithelial cells, leucocytes, and large numbers of streptococci along with the diphtheria bacilli. Clinically, a membrane formation on the conjunctiva may arise from infection of this tissue with organisms other than the diphtheria bacillus; hence, it is wise in all suspicious cases to submit a portion of the membrane to both direct microscopic and cultural examinations.

Infectious Conjunctivitis.

In acute infectious conjunctivitis, various organisms have been found, the most common ones being the Koch-Weeks bacillus, the pneumococcus, and the gonococcus. Occasionally one finds the staphylococcus, streptococcus, colon bacillus, influenza bacillus, Morax-Axenfeld bacillus, the diphtheria bacillus, and other organisms.³

In some regions the Koch-Weeks bacillus is frequently found as the etiologic factor in acute infectious conjunctivitis, while in others it is rarely, if ever, observed. This is an organism of the influenza group, a small, thin, Gram-negative bacillus, which is, so far as known, pathogenic only for the human conjunctiva (see cut). It grows best on media containing a slight amount of human blood, especially in symbiosis with the xerosis bacillus. This latter organism is differentiated from the Klebs-Löffler bacillus only by the application of the fermentation tests spoken of under Examination for Diphtheria Bacilli in the Throat.

The most common bacterial cause of chronic conjunctivitis is the bacillus of Morax-Axenfeld, usually seen as a diplobacillus, several groups of which may at times be arranged in chains (see Plate V). It is a Gram-negative organism and grows well on Löffler's blood-agar, which it digests, forming on the surface at the beginning of its growth, very characteristic small pits.

Gonorrheal Conjunctivitis.

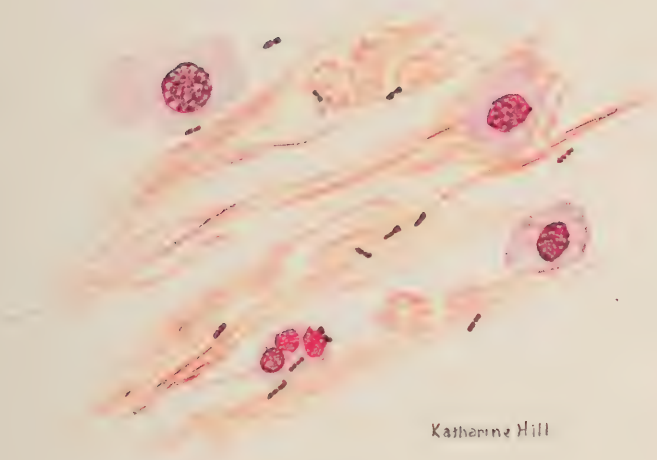
This form of conjunctivitis is much more common than is generally supposed, so that the identification of the organism is of great importance. In the development of this type of conjunctivitis, large or small amounts of pus are invariably present between the folds of the conjunctiva. A portion of this

¹Wilder and McCullough (Jour. Am. Med. Assn., 1914, LXII, 1156) report a case of sporotrichosis of the eye in which the organisms were observed, in direct smears, as Gram-positive oval bodies and in cultures as typical sporothrices. Gifford (Jour. Inf. Dis., 1920, XXVII, 296) finds leptothrices on the conjunctiva and in the meibomian glands and later reports (Arch. Ophthalm., 1920, XLIX, 477) fusiform bacilli in the same locations.

²Begle (Jour. Am. Med. Assoc., 1921, LXXVI, 1301) reports the interesting finding of *filaria loa* beneath the conjunctiva.

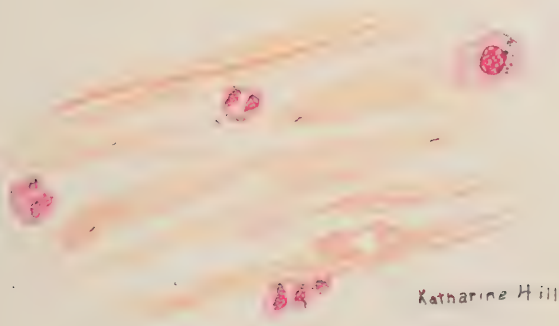
³See Axenfeld, Kolle and Wassermann's Handbuch, 1913, VI, 545, 572 and 587; also, Noguchi and Cohen, Jour. Exper. Med., 1915, XXII, 304; Irons, Brown and Nadler, Jour. Infect. Dis., 1916, XVIII, 315.

PLATE VI.



Katherine Hill

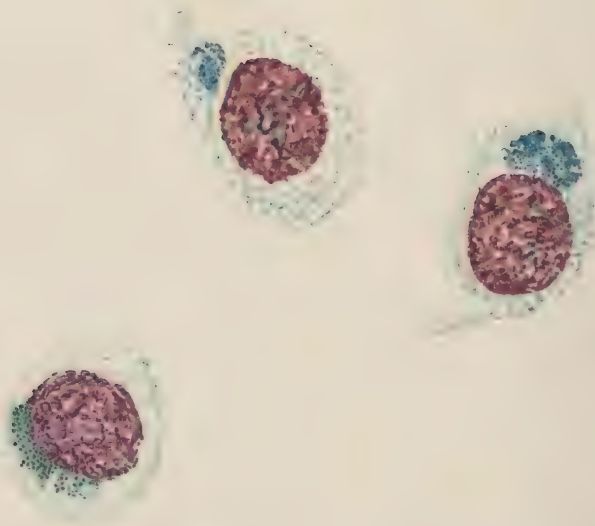
MORAX-AXENFELD DIPLOBACILLUS. (GRAM'S STAIN.)
Courtesy of Dr. Brown Pusey.



Katherine Hill

KOCH-WEEKS BACILLUS. (GRAM'S STAIN.)
Courtesy of Dr. Brown Pusey.

PLATE VII.



Katherine Hill.

TRACHOMA BODIES OF PROWAZEK-GREEFF. (GIEMSA STAIN.)
Courtesy of Dr. Brown Pusey.

pus may be collected by means of a cotton swab or a platinum loop and smeared thinly over a slide. This smear is then fixed in the flame and is stained for the gonococcus by both the methylene blue and Gram stains. The technic of this latter staining process, as well as the characteristic appearance of the organism, will be discussed in a later section.

Trachoma.

Lately, a great deal of attention has been given to some bodies, which are found in trachoma and which are, possibly, the long-sought cause of this infectious disease of the conjunctiva. These organisms are known as the Prowazek-Greeff bodies and are shown in the accompanying cut. They are best stained by the Giemsa stain (see Blood), smears being made in the usual manner.

The present status of these bodies is that they are almost always found in the acute stages of this disease and are, occasionally, observed in other conditions which do not present the clinical features of trachoma. Noguchi and Cohen (*Jour. Exper. Med.*, 1913, XVIII, 572) report the cultivation of an organism, resembling the trachoma body, from cases of trachoma with and without cell inclusions. Whether or not they are the true etiologic factor must still be considered an undecided question.¹

Vernal Conjunctivitis.

An interesting point in the diagnosis of the above condition, as well as of the conjunctivitis of hay fever, is the fact, first observed by Herbert² and confirmed by many, that in these two types of conjunctivitis eosinophile polymorphonuclear leucocytes are found in abundance, whereas in the ordinary forms of conjunctivitis such cells do not obtain. Pusey³ has, therefore, been led to believe that these facts point strongly to a similar etiologic factor, namely pollen, in the vernal conjunctivitis and in the conjunctivitis of hay fever.

¹ See report from the Laboratories of the New York Health Department found in *Proc. New York Path. Soc.*, 1912, XII, 17; Williams and associates, *Jour. Infect. Dis.*, 1914, XIV, 261; Eaton, *Am. Jour. Ophth.*, 1920, III, 422; Nicolle and Guénod, *Arch. de l'Inst. Pasteur de l'Afrique du Nord*, 1921, I, 149.

² *Brit. Med. Jour.*, 1903, II, 73; Bollack, *Presse Méd.*, 1917, XXV, 3

³ *Jour. Am. Med. Assn.*, 1911, LVII, 1207.

CHAPTER III

GASTRIC CONTENTS

I. GENERAL CONSIDERATIONS

The gastric juice is the product of secretory activity of the glands of the stomach. Different series of glands contribute separate elements to the secretion, so that we find much variation, under pathologic conditions, in the composition of this fluid.

The stomach should be regarded as a dilated and specialized portion of the general digestive tube, its walls consisting of the following four coats: mucous, submucous, muscular, and fibrous. From the standpoint of secretory activity the internal or mucous coat is the most important. This mucous membrane is covered throughout its entire length by a single layer of simple columnar epithelium. It follows the various folds or *rugæ* dipping down in places to line the orifices and ducts of the tubular glands which are of such importance in the digestive activity of the stomach. The gastric glands are of two kinds, the peptic or fundus glands, situated in the middle and cardiac thirds of the stomach, and the pyloric glands, found in the pyloric third of the stomach.

Peptic Glands.

These glands are slightly wavy simple tubular depressions, in which a duct, a neck, and a fundus are recognizable. In exceptional cases the fundus is divided, while in nearly all it is tortuous or spiral its extremity being often sharply bent at right angles to the general axis of the tube (Piersol). In these peptic glands are found two types of cell. The first, known as the central, chief or adelmorphous cells, bound the lumen of the gland and form the bulk of the glandular epithelium. These cells are either polyhedral or columnar in form and each contains a spherical nucleus situated within the granular protoplasm. These cells do not stain readily with aniline dyes. The chief function of these central cells of the peptic glands is to secrete the rennin and lipase which are present in the gastric juice. The second type of cell in the peptic gland is known as the parietal, acid or oxyntic cell and is situated in the periphery of the gland immediately below the basement membrane. These cells are more oval or angular in form, are larger than the chief cells, are more finely granular in structure and stain deeply with the aniline dyes.

The Pyloric Glands.

These glands are characterized by their relatively long wide ducts into which the several divisions of the body open; the tubular compartments are wavy and tortuous and frequently end in slightly expanded extremities. The duct is lined by tall columnar epithelium, the cells becoming lower and broader as they approach the neck and toward the fundus. The cells contain finely

granular protoplasm and do not secrete mucus but a viscous, tenacious albuminous liquid of alkaline reaction (РН 7-7.5.)¹ Parietal or acid cells do not occur in the pyloric gland, being confined to the true peptic gland (Piersol).

It will thus be seen that the active portions of the gastric juice are secreted by the fundus glands, the pyloric glands contributing nothing except a small amount of the ferments and liquid portion, the mucus being largely derived from the goblet cells which line the entire stomach and the wider portion of the glandular ducts. These ferments do not exist in the cells as such, but, rather in the form of zymogens or prozymogens which become active only in the presence of the free hydrochloric acid.²

The free hydrochloric acid of the gastric juice is formed in the parietal cells of the peptic gland.³ The mechanism of this formation is not absolutely established, but it seems probable that this free acid arises from the chlorids taken up from the blood by these cells.⁴ Just what is the active agent in causing the conversion of the chlorids into free acid seems to be in doubt, but it may be either the continuous action of carbonic acid or, as Maly assumes, the interaction of the sodium phosphate (Na_2HPO_4) with the chlorids of the cell. It is also probable that the osmotic influences may be very great in the production of this free hydrochloric acid as Koepe advocates.⁵ This acid is present at all times in the normal stomach, being found even in cases of extreme starvation.

The careful work of Pawlow⁶ has shown that various factors influence the quantity and quality of the normal gastric juice. He asserts that the "appetite is the first and mightiest exciter of the secretory nerves of the stomach, a factor which embodies in itself a something capable of impelling the empty stomach of the dog in the sham feeding experiment to secrete large quantities of the strongest juice. A good appetite in eating is equivalent from the outset to a vigorous secretion of the strongest juice; where there is no appetite this juice is also absent." Moreover, under natural conditions, the stimulation of food is a very important factor. The administration of a diet causes a secretion of gastric juice which is directly proportionate, both in amount and activity, to the diet taken. We find, according to Chigin, that the greatest digestive power is shown by the juice excreted after the administration of bread, although the total acidity is greatest following an intake of meat. If we compare equivalent weights of food material we find that flesh requires

¹ See Ivy and Oyama (*Am. Jour. Physiol.*, 1921, LVII, 51).

² A secretagogue, called by Edkins gastrin, is believed to be formed by contact of certain substances with the gastric mucosa. This gastrin apparently causes a true gastric secretion. See Keeton and Koch, *Am. Jour. Physiol.*, 1915, XXXVII, 481; Luckhardt, Keeton, Koch, and La Mer. *Ibid.*, 1920, L, 527; Keeton, Koch and Luckhardt, *Ibid.*, 1920, LI, 454 and 469; *Ibid.*, LII, 508.

³ See Harvey and Bensley, *Biol. Bull.*, 1912, XXIII, 225; also López-Suárez, *Biochem. Ztschr.*, 1912, XLVI, 490; Hammett, *Anat. Rec.*, 1915, IX, 21. Bergeim, *Proc. Soc. Exper. Biol. and Med.*, 1914, XII, 21.

⁴ See Leist, *Wiener Arch. f. iun. Med.*, 1921, II, 491; Rosemann, *Arch. f. d. ges. Physiol.*, 1921, CXC, 1.

⁵ See Kelling, *Arch. f. Verdauungskr.*, 1920, XXVI, 287.

⁶ *The Work of the Digestive Glands*. London, 1902. See, also, Wolfsberg, *Ztschr. f. physiol. Chem.* 1914, XCI, 344; Miller, Bergeim, Rehfuß and Hawk, *Am. Jour. Physiol.*, 1920, LII, 1. Dupuy, *Paris Méd.*, 1920, X, 186; Heyer, *Arch. f. Verd.-Kr.*, 1921, XXVII, 227.

the most gastric juice and milk the least; but taking equivalents of nitrogen, bread needs the most and flesh the least. In this connection it is well to remember that the gastric secretion varies from hour to hour. Thus the most active juice occurs with flesh in the first hour, with bread in the second and third hour, and with milk in the fifth to the sixth hour. The point of all this is that the rate and time of secretion of the gastric juice is always characteristic for each diet. Even water has been indisputably shown to be particularly potent as a secretory stimulus.¹

Moreover, it has been found that the hydrochloric acid first secreted combines at once with the proteins of the various food stuffs, so that we may find no free hydrochloric acid in the gastric contents, although the secretion may be normal and may show a very high degree of total acidity.

II. METHODS OF OBTAINING THE GASTRIC CONTENTS

Unless the patient is one who can easily eject the contents of the stomach by vomiting, it is necessary to resort to the introduction of the so-called stomach-tube for the removal of the contents.

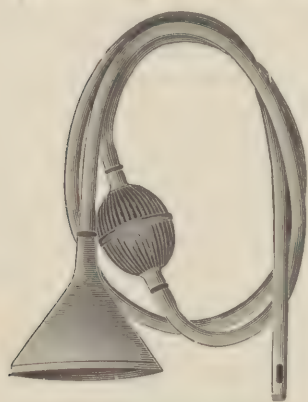


FIG. 14.—Stomach tube.

This stomach-tube consists of a long, soft rubber tube about 75 cm. in length, having a lumen 6 to 7 mm. in diameter and provided with either two oval lateral openings or with three, one being at the end of the tube.² Before introduction of the stomach-tube, it should be moistened with warm water and should be thoroughly cleaned.

Introduction of the Tube.

The patient must be in a sitting posture, a towel or a rubber sheet being placed about his neck to prevent soiling of the clothes with the saliva or material which is occasionally brought up during the passage of the tube.

False teeth should be removed and anything interfering with the passage of the tube should be avoided. In patients who are hypersensitive, a 10 per cent. solution of cocain is applied to the pharynx. The head of the patient is now bent slightly forward, never backward as some advise, and the mouth slightly opened, care being taken never to use a depressor on the tongue. The rubber tube, held as one would a pen, is passed gently backward over the tongue until its tip strikes the posterior wall of the pharynx, when it runs

¹ With reference to the gastric response to different articles of diet introduced into the stomach see Bergeim, *Am. Jour. Physiol.*, 1917, XLV, 1; Bergeim, Evvard, Rehfuß and Hawk, *Ibid.*, 1919, XLVIII, 411; Fishback, Smith, Bergeim, Lichtenthaler, Rehfuß and Hawk, *Ibid.*, XLIX, 174; Smith, Fishback, Bergeim, Rehfuß and Hawk, *Ibid.*, 204 and 222; Miller, Fowler, Bergeim, Rehfuß and Hawk, *Ibid.*, 254; *Ibid.*, 1920, LI, 332; Rehfuß, *Am. Jour. Med. Sc.*, 1920, CLX, 428; Miller, Bergeim and Hawk, *Science*, 1920, LII, 253; Miller, Bergeim, Rehfuß and Hawk, *Am. Jour. Physiol.*, 1920, LII, 1, 28 and 248; *Ibid.*, LIII, 65; Sutherland, *Ibid.*, 1921, LV, 258; Bennett & Dodds, *Brit. Jour. Exp. Path.*, 1921, II, 58; Hoffmann and Rosenbaum, *Jahrb. f. Kinderhke.*, 1922, XCVII, 40.

² In this connection see Togami, *Jour. Lab. & Clin. Med.*, 1919, V, 178; Mac Intyre, *Glasgow Med. Jour.*, 1920, XCIV, 101; Mizell, *Southern Med. Jour.*, 1920, XLII, 96; Lyon, Bartle and Ellison, *New York Med. Jour.*, 1921, CXIV, 272; Butsch and O'Brien, *Jour. Lab. & Clin. Med.*, 1922, VII, 431.

downward and may be readily introduced into the stomach, by slight forcing. As the tube reaches the esophagus, many patients complain of a sense of suffocation, which is not real but apparent. The tube interferes in no way with the normal respiratory movements and hence the patient should be cautioned to breathe normally and not forget to breathe. If the patient will swallow normally, the passage of the tube is greatly facilitated. It occasionally happens that highly nervous patients have great difficulty in swallowing this tube, so that it may be necessary to defer the withdrawal of the contents to a second or even a third period. It is never wise to excite a patient by forcing matters at any stage of the investigation. If any sign of cyanosis or marked pallor is evident the tube should be immediately withdrawn and a second attempt made at some later time.¹ When the tube has reached the floor of the stomach, which is in normal cases about 40 cm. from the incisor teeth, a distinct resistance to further passage of the tube will be noticed. This point



FIG. 15.—Turk's aspiration apparatus.

should be carefully observed as the forcing of the tube beyond this point may produce rupture of the stomach wall or may cause the tube to "buckle."² In this latter condition it will be impossible to withdraw the stomach contents. Many of the tubes used for gastric examination have a mark indicating the normal length of tube from the incisor teeth to the stomach wall, so that one has a definite idea when he has introduced the tube into the right point. In some cases the gastric juice will commence to flow from the tube as soon as it is properly introduced, but in the majority of cases some help is necessary to start the siphonage. Frequently all that is needed is to ask the patient to bear down with his abdominal muscles or to cough a little. In other cases aspiration is necessary. One may employ an ordinary Politzer bag or a Boas bulb for starting the fluid in the tube. This is very readily done by compressing the bulb and applying it, while compressed, to the end of the tube in such a way that the suction will be sufficient to draw the material into the tube. Once started, the material flows quite readily, but it may be necessary to use aspiration several times as the tube may become clogged.

¹ McKinlay (*Jour. Am. Med. Assoc.*, 1921, LXXVI, 431) calls attention to the increase of blood pressure associated with the passage of the tube.

² In this connection see Harmer and Dodd, *Arch. Int. Med.*, 1913, XII, 488.

If it is desired to wash out the stomach, either to obtain the total gastric contents or for the purpose of mere lavage, a funnel is attached to the external end of the stomach-tube and about 500 c.c. of water are allowed to flow through the tube into the stomach. In this operation the funnel is held either on a level with the patient's mouth or a very little bit above. By depressing and inverting the funnel over a suitable vessel, before all the water has left it, return flow will soon set in and the stomach will be practically emptied by siphonage. In some cases it becomes necessary to add more water, but in no case should any be added after the patient complains of a feeling of distress.

In collecting the stomach contents one should avoid as far as possible any admixture with the saliva which is more freely excreted at this time than normally. This is best done by wrapping a cloth about the tube so that the material may be absorbed as it runs along the side of the tube. After one has obtained the gastric contents, the tube is compressed with the fingers and is rapidly withdrawn, care being taken to keep up the compression so as to hold in the tube material which has not already passed into the receiving vessel.

In cases in which water has been introduced to wash out the stomach after the gastric contents have been obtained, one should be careful to note the amount of fluid poured into the stomach so that he may be able to judge of the amount again received.

Not every case with which the practitioner meets is amenable to such manipulation. We find as especial contraindications uncompensated valvular lesions of the heart, arteriosclerosis, aneurysm, advanced pulmonary tuberculosis, marked emphysema, acute febrile diseases, severe hemorrhage, especially from ulcer or carcinoma, and excessively developed nervous antipathy.

Fractional Examination of the Gastric Secretion.

Recognizing that the passage of the ordinary stomach tube is, oftentimes, disagreeable to the patient and that there are, also, certain contraindications to its use and certain possible inaccuracies in the results obtained by the usual methods of withdrawal of the gastric contents, Rehfuess was lead to devise a modification of the Einhorn duodenal tube employing a special metal tip, which is slotted instead of perforated, the slots being so cut that their diameter is as great as the caliber of the rubber tubing used. This tip is made of steel or manganese bronze, is bulbous in shape, weighs 90 to 120 grains, and is attached to the usual No. 8 French tubing. The shape of the tip permits of it being swallowed easily and its weight carries it readily down into the stomach with little inconvenience to the patient. Further, it is possible to leave it in the stomach for long intervals, so that the investigator may withdraw the gastric contents at as frequent intervals as he may desire.

By the introduction of this Rehfuess tube, it is possible to follow the entire cycle of gastric digestion by withdrawing, at any given moment, any or all of the contents. To stimulate gastric secretion Rehfuess and his coworkers adopt the Ewald or a similar test meal (see below), about 2 ounces of the liquid portion being reserved to wash down the tube. At the close of the

meal, which should consume about 10 minutes, the tube is swallowed and left in position, the patient being asked to read or to amuse himself in some such way, as he suffers little or no inconvenience from the presence of the tube. If the patient will not voluntarily swallow the Rehfuß tube, the procedure of Hertz¹ may be followed: The end of an ordinary Ewald gastric tube is cut off and the Rehfuß tube inserted therein so that its tip projects. The "double" tube is then inserted as described above, after which the Ewald tube is withdrawn leaving the Rehfuß tube *in situ*.

At intervals of 15 minutes small portions, not exceeding 10 c.c. of the gastric contents are aspirated by gentle suction, until the close of digestion, which point is indicated by (1) failure to aspirate any further material; (2) the character of the preceding specimens; and (3) by lavage, which permits of the determination of the presence of any food residues and their quantity.

This method is based on the assumption that the gastric contents, after the test meal, is a homogeneous mixture, so that a sample withdrawn in the manner stated represents the acid concentration of the contents as a whole. The acidity (determined as outlined below) of the various specimens withdrawn may be plotted in the form of a curve. From the work of Rehfuß and his associates, as well as of various others, it is evident that there is no such thing as a specific isolated curve, deviation from which indicates pathologic conditions. In the interpretation of these curves, Rehfuß regards the following phases as important: (1) The period of "ascension." This usually occupies the first 30 minutes and indicates the rapidity and intensity of the response to a known stimulus; (2) the character and height of the high point or "acme," whether (a) accelerated or (b) retarded and whether (a) abrupt or (b) sustained; (3) period of descent or decline; and (4) character and modification of the food residues.

Normal healthy individuals appear to react to the Ewald meal in one of three ways (1) The "isosecretory" type shows a steady rise, high point, in terms of N/10 alkali, 60, usually sustained for from $\frac{1}{2}$ to 1 hour and then a gradual decline with total disappearance of food residues in from 2 to $2\frac{1}{2}$ hours. The curve is usually steady and unbroken, its high point is usually rounded and not abrupt and is to be found in the neighborhood of 1 hour; (2) the "hypersecretory" type shows a rapid response to stimuli, often a marked change in the acidity, even of the 5 minute specimens, rapid increase in acidity, high point from 70 to 100 or over, either sustained or abrupt, and a slow decline or none at all in the usual time; (3) the "hypersecretory" type is similar to the first but there is usually a slower ascent, slower response to stimuli and a high point from 40 to 50, digestion being completed in 1 to $2\frac{1}{2}$ hours. Rehfuß has shown that, even though the same figures may not obtain on succeeding days in the same healthy person under the same conditions, the type of curve will be identical, although Kopeloff, working with cases of functional psychoses, reports that the curves from the same individual, within a short period of time, vary as much from one another as the difference between the curves of different individuals.

¹ Jour. Am. Med. Assoc., 1922, LXXVIII, 651.

It is clear that gastric analysis has three important functions: (1) Determination of evacuation time or motor activity; (2) determination of secretory activity and work; and (3) the determination of the presence of pathologic products, such as pus, blood, bacteria, etc. This method of Rehfuß would seem to be of great advantage in the study of gastric function, as it offers a much wider field of study than does the older method and affords a more nearly correct idea of the conditions actually prevailing in the stomach during the cycle of digestion. In this connection it is to be said, however, that the work of Gorham and of Wheelon seems to offer some food for thought. They show that different portions of the gastric chyme may vary widely in acid concentration; and, therefore, a small sample, as obtained by the Rehfuß method, is not truly representative of the gastric chyme as a whole, because the acid concentration of this portion may vary considerably from the highest or lowest acidity of some remaining portion or the average acidity of the entire gastric contents of that period. The sample obtained by the fractional method represents only the acidity of the gastric chyme at that moment in the part of the stomach from where it is obtained; or in, other words, it is dependent entirely on the position of the tip of the tube in the stomach, which position is necessarily a constantly changing one, owing first to the change in size and position of the stomach while emptying itself through the pylorus and by aspiration; secondly, to the shortening and lengthening of the stomach from gastric contractions; and thirdly, to the peristaltic waves that tend to carry the tube toward the pylorus. Gorham, therefore, insists that the stomach must be completely emptied at a definite time.¹

Test Meals.

As the secretion of the gastric juice is so dependent upon administration of food, it has become the custom to use certain combinations of food principles, which will excite gastric activity and enable us to obtain a juice which will give us more or less definite ideas of its composition in the condition investi-

¹ See Rehfuß, *Am. Jour. Med. Sc.*, 1914, CXLVII, 848; Rehfuß, Bergeim, and Hawk, *Jour. Am. Med. Assoc.*, 1914, LXIII, 11 and 909; Rehfuß and Hawk, *Ibid.* 2088; Bergeim, Rehfuß, and Hawk, *Jour. Biol. Chem.*, 1914, XIX, 345; Rehfuß, *Jour. Am. Med. Assoc.*, 1915, LXIV, 509; Clarke and Rehfuß, 1737; Foster and Hawk, *Jour. Am. Chem. Soc.*, 1915, XXXVII, 1347; Spencer, *Jour. Biol. Chem.*, 1915, XXI, 165; Rehfuß, *Am. Jour. Med. Sc.*, 1915, CL, 72; Fowler, Rehfuß and Hawk, *Jour. Am. Med. Assoc.*, 1915, LXV, 1021; Smith, Miller, and Hawk, *Jour. Biol. Chem.*, 1915, XXIII, 505; Hawk, *Am. Jour. Physiol.*, 1916, XXXIX, 450; Talbot, *Jour. Am. Med. Assoc.*, 1916, LXVI, 1840; Best, *Ibid.*, LXVII, 1083; Fishbaugh, *Ibid.*, 1275; Wilensky, *Ibid.*, 1917, LXVIII, 891; Crohn and Reiss, *Am. Jour. Med. Sc.*, 1917, CLIV, 857; Robinson, *Arch. Int. Med.*, 1917, XIX, 220; Fowler, *Jour. Biol. Chem.*, 1917, XXXII, 380; Cessna and Fowler, *Ibid.*, 1919, XXXIX, 25; Best, *Am. Jour. Med. Sc.*, 1920, CLX, 880; Bennett and Ryle, *Brit. Med. Jour.*, 1920, II, 503; Bennett and Venables, *Ibid.*, 662; White, *Journal-Lancet*, 1920, XI, 621; Rehfuß and Hawk, *Jour. Am. Med. Assoc.*, 1920, LXV, 440; Cole and Adie, *Lancet*, 1921, I, 423; Upham, *Jour. Am. Inst. Homeopathy*, 1921, XIII, 622; Ryle, *Guy's Hosp. Rep.*, 1921, LXXI, 42, 45, 158, and 442; Bennett and Ryle, *Ibid.*, 286; Gorham, *Arch. Int. Med.*, 1921, XXVII, 434; Cordero, *Rev. Mexicana de Biol.*, 1921, I, 140; Levy, *Texas State Jour. Med.*, 1921, XVI, 483; Chiasserini, *Policlinico*, 1921, XXVIII, 647; Rehfuß and Hawk, *Jour. Am. Med. Assoc.*, 1921, LXXVI, 371 and 564; Lermann, Rehfuß and Hawk, *Ibid.*, 1340; Fowler, Spencer, Rehfuß and Hawk, *Ibid.*, LXXVII, 2118; Lyon, Bartle and Ellison, *New York Med. Jour.*, 1921, CXIV, 272; Wheelon, *Arch. Int. Med.*, 1921, XXVIII, 613; Kopeloff, *Jour. Am. Med. Assoc.*, 1922, LXXXVIII, 404; Fitz, *Ibid.*, 1446; Kopeloff, *Jour. Inf. Dis.*, 1922, XXX, 613.

gated.¹ It must be remembered that marked idiosyncrasy toward certain foods exists, so that we may not use in all cases the same sort of a diet for exciting the gastric juice. The results obtained in pathologic conditions are compared with those obtained from normal individuals under the influence of the same diet. In this way we are able to say, with some degree of certainty, that a suspected case shows normal or abnormal gastric relations. These diets, the so-called test meals, are always given to the fasting stomach and are removed after a suitable time by the use of the stomach-tube. The time best suited for the administration of these meals is in the morning, as the stomach has had occasion during the night to empty itself of most of its contents.

Ewald Test Meal.

This meal, which is, perhaps, the most frequently employed in general work, consists of a roll or piece of bread or toast without butter and two cups of water or tea² without milk or sugar. In approximate figures this will represent 35 grams of wheat bread and 400 c.c. of water or tea.³ The bread should be well masticated so that the later withdrawal of the contents may not be interfered with by the plugging up of the openings in the tube. The contents are removed one hour later and consist normally of 30 to 50 c.c., depending both upon the skill of the operator and upon the condition of the stomach. Hypermotility of the stomach will diminish the quantity of contents received, while a hypomotility will increase the quantity.

Boas Test Meal.

This meal consists of a dish of oatmeal prepared by concentrating to 500 c.c. a liter of water to which a tablespoonful of oatmeal is added. This meal was advised to prevent the introduction into the stomach of lactic acid which is a normal constituent of bread. While this small amount of lactic acid introduced in the Ewald meal has little significance, yet in doubtful cases it is well to avoid it. The contents of the stomach are withdrawn one hour later when the amount may be very small. If the stomach shows normal digestive powers most of the material will be then passed into the intestine, while an appreciable amount of material would indicate either a dilatation of the stomach or pyloric obstruction.

Riegel Test Meal.

This test meal has the advantage of permitting the patient to use a diet which is more normal than either of the ones previously mentioned. This

¹ See Smithies, *Am. Jour. Med. Sc.*, 1915, CXLIX, 183; also, Heaton, *Brit. Med. Jour.*, 1915, I, 710.

² Kober, Lyle and Marshall (*Jour. Biol. Chem.*, 1910, VIII, 95) have shown that it is almost impossible to detect blood in the presence of tannic and gallic acids, as found in tea. It is wise, therefore, not to use tea in the test meal when blood is suspected. Bergeim, Reh-fuss and Hawk, *Jour. Biol. Chem.*, 1914, XIX, 345, show the direct stimulatory power of water. Brendle (*Med. Klin.*, 1916, XII, 1257) advocates a test meal of 100 c.c. of water. Dock substitutes 1 shredded wheat biscuit for the toast of the Ewald meal.

³ Roberts (*Jour. Am. Med. Assn.*, 1912, LVIII, 753) adds to this breakfast 30 grams of lactose. He extracts the meal as usual and then washes out the stomach with 200 c.c. of water. The total gastric juice excreted and the acidity when excreted are calculated by the method of Mathieu (p. 58).

diet is more important in America, where we are not accustomed to the continental breakfasts, than it is in Germany where the Ewald and Boas meals are more usual.

The Riegel meal is given in the middle of the day at a time when the patient is accustomed to such a meal. It consists of about 400 c.c. of soup, 200 grams of beef-steak, and either two slices of white bread or 150 grams of mashed potato along with one glass of water. This meal is withdrawn at the end of three to four hours. It has the advantage of allowing us to judge of the length of time which the food remains in the stomach under normal conditions and, also, to form an opinion of the rate and amount of digestion which has taken place. This meal incites a more nearly normal gastric juice than does the Ewald or Boas meal, but it is such that clogging of the stomach tube by particles of undigested food frequently occurs.

Fischer Test Meal.

This meal, introduced by an American physician, has the advantage of more nearly approaching an American breakfast than the others. It consists of the bread and tea of the Ewald meal along with a quarter of a pound of finely chopped lean beef broiled and seasoned. The contents are removed at the end of three hours.

Salzer Test Meal.

This is in reality a double meal and is given as follows: For breakfast the patient receives 30 grams of lean cold roast meat, finely chopped, 250 c.c. of milk, 60 grams of rice, and one soft-boiled egg. Four hours thereafter a second meal is given, consisting of 35 to 70 grams of stale wheat bread and 400 c.c. of water. The contents are then removed one hour after this second meal. Under normal conditions of digestion and motility the stomach contents should show no remnants of the first meal.

Sahli Test Meal.

This meal was introduced to enable the worker to examine quantitatively the material withdrawn. The inconstant composition of the ordinary test meals makes it rather difficult to judge of the digestive power of the stomach. Sahli has introduced, therefore, a soup prepared as follows: Twenty-five grams of flour and 15 grams of butter are placed in a pan and browned over a fire. Three-hundred and fifty c.c. of water are then added and the whole boiled for five minutes (the loss in volume being replaced by fresh water) after which it is seasoned with a little salt. In this soup the fat is in the form of a very fine emulsion and the taste is so pleasant that a more nearly normal stimulus to gastric secretion is offered. The patient is now allowed to take 300 c.c. of this soup, while the remaining 50 c.c. are retained for a determination of the fat content. The contents are withdrawn, one hour after the meal, from the stomach which must have been thoroughly washed out prior to the administration of the meal.¹

We then determine the absolute amount of fat remaining in the stomach

¹ Sahli (*Lehrbuch d. klin. Untersuchungsmethoden* 6 Aufl., 1913, I, 656) apparently now prefers a soup prepared from Maggi or Teston bouillon and yolk of egg instead of the above.

after the test digestion and compare this amount with that introduced. As we cannot be sure that the entire stomach contents have been withdrawn, we must know the residual amount of gastric juice. For this purpose one resorts to the method of Mathieu, which will be discussed later (p. 64).

The amount of fat both in the original soup and in the withdrawn stomach contents is then determined and the total gastric juice calculated. This method of fat determination will be given in detail under Milk, to which the reader is referred.

Calculation of Results.

"The following calculations are possible from a consideration of the residue from the acidity of the gastric filtrate, and from the difference between the amount of fat found in the ingested flour soup and that found in the expressed contents.

"By the addition of the value X , found in the calculation of the residue (p. 64), to the amount of contents expressed after one hour, there is obtained the volume of the contents which were actually present in the stomach at the end of that period. This we designate as To . From the absolute fat-content of To , there can be determined how much of the volume can be ascribed to the ingested flour soup. The amount of fat remaining in the stomach serves, therefore, as a measure for the amount of soup remaining. This is designated as Su . Representing this mathematically, we have the proportion $To : Su : F : f$, in which F represents the percentage fat content of the soup and f that of the expressed contents. $To - Su$ will give, of course, the volume of gastric juice in the expressed contents. If the acid-content of To has been determined, it is possible from these data to proceed further and to calculate what acidity was possessed by the pure gastric juice as it was excreted. Thus if 75 c.c. of pure gastric juice are present in the stomach contents, whose volume amounts to 150 c.c. with 2 per cent. acidity, then the acid-content of the pure gastric juice is evidently 4 per cent. The determination of this acidity may be expressed by the proportion, $To : A :: Ma : a$, in which To represents the amount of expressed contents including the residue, Ma the amount of secretion contained in the expressed contents ($To - Su$), A equals the acidity of the pure secretion in per cent, and a the percentage acidity of the expressed contents" (Sahli).

It is important to remember in selecting a test meal for any given case that the tastes of no two persons are alike and that no two persons will react identically toward the stimulation of the same test meal. The results can only be comparative and have, in this sense, some value. Too much rigidity in administration of such meals will lead frequently to mistakes in diagnosis, so that one should learn to vary his test meals rather than to rely upon a single one in all cases. Another point to be borne in mind is that the meals should be removed at the time of optimum secretion, which may not in all cases be at the end of one hour with the Ewald meal.¹ "The technic commonly employed for estimating gastric function is entirely inadequate, inasmuch as it

¹ See Ettinger, Internat. Beitr. z. Ernährungsstör., 1913, IV, 454. Frenkel-Tissot, Cor.-Bl. f. Schw. Aertzte, 1919, XLIX, 1423.

indicates but one phase in a constantly changing cycle, and that phase is by no means always the high point in the digestive curve" (Rehfuss, Bergeim and Hawk).

III. MACROSCOPIC EXAMINATION

The gastric juice is a clear, colorless, easily filtered, levorotatory fluid having a distinctly acid reaction, an acid taste, and a characteristic odor. Its specific gravity, when the stomach is empty, ranges between 1004 and 1006.5; after the ingestion of food from 1010 to 1020 and more than 1020 when the production of acid is diminished (Landois). Its cryoscopic point is -0.38 degree to -0.444 (Roth and Strauss).

Amount.

The figures for the total amount of gastric juice secreted in 24 hours are variable. Carlson¹ shows that an adult secretes a total of about 1500 c.c. in 24 hours, appetite being a potent factor in this process. The amount of fluid obtained one hour after an Ewald meal is from 20 to 50 c.c., although larger amounts ranging from 200 to 500 c.c. indicate either diminished motility or hypersecretion, on the one hand, or dilatation associated with pyloric stenosis, on the other. It is to be remembered that the quantity of juice secreted is influenced by the appetite and by the amount and character of the food taken, as well as by the age and sex of the patient and the time of day at which the food is taken. The largest amounts of gastric juice are found in cases of hypersecretion when it is not uncommon to find a liter or more of gastric juice in the non-digesting stomach.

In order to determine the total amount of gastric juice secreted, one cannot rely upon the quantity removed as there is always a slight residuum. The method of Mathieu and Rémond is commonly used for such determinations. This gives results very nearly exact, at least for clinical purposes. With this method the gastric contents are removed, as nearly as possible, after an Ewald meal. A definite quantity of water, usually 300 c.c., is then poured into the stomach through the tube and is thoroughly mixed by moving the funnel up and down and by pressure upon the stomach. As much as possible of this added fluid and the remaining gastric juice is removed and collected in a separate vessel. The acidity of the undiluted as well as of the diluted stomach contents is then determined by titration. From the difference in these two values conclusions may be drawn as to the degree of dilution and to the residual amount of stomach contents which was not expressed. The amount expressed plus the residual amount equals the total gastric contents.

The following is the method of calculation according to Mathieu:

Let a = acidity of the undiluted gastric contents.

Let b = acidity of the diluted gastric contents.

Let x = amount of the test meal remaining in the stomach after expression.

Let 300 c.c. = the amount of water introduced into the stomach for dilution. Then

¹ Am. Jour. Physiol., 1915, XXXVII, 50.

$$\begin{aligned} a : b &:: x + 300 : x \\ ax &= b(x + 300) \\ x &= \frac{300b}{a - b} \end{aligned}$$

An absolutely accurate result, in the study of gastric activity, can be obtained only when the total quantity of gastric juice is known.¹ It is, therefore, necessary in stating, for instance, the acidity of a stomach contents to calculate the total available acidity rather than the mere degree of acidity. It is self-evident that a stomach contents expressed in the ordinary way, which shows an acidity of 40°, may have this acidity in a total quantity of 50 c.c., while an acidity of 40°, with a total quantity of 200 c.c., would represent actually four times as much hydrochloric acid available. It would seem, therefore, to the writer that the method of representing acidity in terms of degrees without any reference to the amount of gastric contents is absolutely irrational.

Color.

Gastric juice is normally a practically colorless liquid, although at times it may be somewhat opaque and, therefore, much whiter in color. Variations in this colorless fluid are observed after test meals due to admixture of various food products, so that we may have distinctly brownish colorations due to the tea or particles of toasted bread, while in the test meals consisting of meat the color may be more of a reddish tone.

Pathologically, we may find a distinct red color due to the presence of blood. This bright red color comes from the presence of fresh blood from a hemorrhagic gastric ulcer or may be derived from abrasions of other portions of the alimentary tract. If the blood has been thoroughly mixed with the stomach contents for some time it may appear in the form of a brownish-black deposit, the so-called coffee-ground material. The blood in these cases is in the form of hematin and must be tested for as later outlined. This coffee ground appearance is particularly evident in cases of gastric carcinoma.

The color of the gastric contents may be either a yellow or a green, due to the presence of bilirubin in the former case and biliverdin in the latter. This biliary pigment should be detected by the tests outlined under Urine. The presence of bile in the gastric contents is indicative of duodenal occlusion.

In cases of intestinal occlusion below the duodenum we occasionally find fecal matter in the gastric contents. This is characterized by the brownish-black coloration and by its intense odor.

Odor.

The normal gastric juice is practically odorless or very slightly sour. Admixtures of material coming from the intestines cause a very intense odor, while the material rising from abscesses along the alimentary tract above the stomach will frequently give rise to a very offensive odor. In the vomitus obtained under various pathological conditions the odor may be very characteristic. Thus, in uremia we may find a distinct odor of ammonia, in alcohol

¹ See Rehfuess, Jour. Am. Med. Assn., 1915, LXIV, 569.

intoxication a distinct alcoholic odor is evident, while in cases of stagnation of gastric contents an intensely strong odor is observed. In cases of dilatation we frequently find the organic acids so much increased in amount that distinct odors are noticeable.

Consistency.

The normal stomach contents are usually watery in character, but may vary due to admixture with extraneous material. After test meals or following a vomiting spell we may find portions of unchanged protein or carbohydrate material. The amount of bread taken with an Ewald meal should be so far digested in one hour as to form a puree-like mass which settles out on standing. Various food residues are, of course, present in the vomitus so that the consistency and appearance may give us much valuable information regarding the digestive process. In cases of mucous catarrh or in those showing either a diminution or an increase in the amount of hydrochloric acid we may find after a test meal the presence of large amount of tough, slimy, mucoid material, which may be so abundant as practically to make it impossible to filter the contents. The consistency of such material may be almost that of a paste, or may be simply that of a thick syrup which on pouring from the vessel onto the filter will form distinct mucoid threads. The presence of an increased amount of mucus is of some diagnostic importance and should, therefore, be looked for under all circumstances.

Gastric Contents from Fasting Stomach.

The stomach is practically never empty, always containing a certain amount of acid fluid.¹ Observations by Rehfuß and his associates would indicate that the normal gastric residuum averages about 50 c.c. Anything above this amount would mean either motor insufficiency or hypersecretion. One may differentiate these two conditions by washing out the stomach at night, when the material withdrawn in the morning will be extremely scanty if the condition is one of motor insufficiency.

This fluid from the fasting stomach is thin, has a specific gravity of 1004 to 1005 and an average total acidity of 30, contains about 17 degrees of free hydrochloric acid, no lactic acid, and no bacteria. It is very commonly bile-stained, may be alkaline from the presence of pancreatic juice and may contain large amounts of mucus. As such material is always found in the fasting stomach it is well to make it a rule to wash out the stomach the night before giving a test meal.

Vomitus.

In those cases which are associated with frequent vomiting we may obtain

¹ See Carlson, *Am. Jour. Physiol.*, 1915, XXXVII, 50; also, Rehfuß, Bergeim and Hawk *Jour. Am. Med. Assn.*, 1914, LXIII, 11 and 909; Fowler, Rehfuß and Hawk, *Ibid.*, 1915, LXV, 1021; Fowler and Zentmire, *Ibid.*, 1917, LXVIII, 167; Raymond and Robert, *Bull. soc. med. hôpitaux de Paris*, 1918, XLII, 1134; Pron, *Bull. Acad. de Méd.*, 1920, LXXXIII, 361; Jarno and Hecks, *Wiener, klin. Wchnschr.*, 1920, XXXIII, 578; Hayem, *Bull. soc. med. hôpitaux de Paris*, 1920, XLIV, 1523, 1500 and 1680; Leist and Weltmann, *Wiener Arch. f. inn. Med.*, 1921, II, 245; Poppens, *Am. Jour. Méd. Sc.*, 1921, CLXI, 203; Jarno and Vándorfy, *Arch. f. Verd.-Kr.*, 1921, XXVII, 364; Pron, *Bull. Acad. de Méd.*, 1921, LXXXV, 353; Rehfuß and Hawk, *Jour. Am. Med. Assoc.*, 1921, LXXVI, 564; Lermann, Rehfuß and Hawk, *Ibid.*, 1340.

much valuable information from the examination of the ejected material. The amount of material vomited will depend, of course, upon the motility of the stomach. In cases of dilatation or of stenosis we frequently find two or three quarts of material, while in conditions associated with hypermotility we may have simply a scanty highly mucoid vomitus. The presence of food particles will give much information as to the digestive power of the stomach. If undigested meat fibers are found in the vomitus, ejected three hours after eating, one may assume more or less disturbance of protein digestion. If particles of unchanged bread are found, three hours after taking, the disturbance in protein digestion is probably more marked than in the case of the meat fibers. If an individual vomits bits of food more than seven hours after a meal, some impairment of motility must exist, according to Sahli, for after that interval even a hearty meal should have completely left the stomach. The vomiting of an acid liquid containing no food particles is quite characteristic of hypersecretion of gastric juice.

The degree of acidity of the vomitus as well as the amount of hydrochloric acid present very frequently enables us to judge of the activity of the juice. These figures will not be as reliable as are the ones obtained after a test meal, but may serve in cases in which the stomach-tube cannot be passed.

Frequently one finds a vomitus which is quite foamy and smells strongly of the volatile fatty acids. In such conditions we may assume a diminution in the amount of hydrochloric acid, which normally prevents the occurrence of any such decomposition, or we may ascribe this condition to simple stagnation of the gastric contents. Such contents will show microscopically the presence of large numbers of *sarcinæ ventriculi*, yeast fungi, and various bacteria.

The blood in the vomitus varies from a slight streaking of the material to a fluid which shows intimate mixing with the gastric contents. In cases of recent hemorrhage, which is particularly common in ulcer of the stomach, an abundant admixture of fresh arterial blood or of dark coagulated blood is observed. Brown or black coffee-ground-like material is particularly suggestive of carcinoma, although the same condition may result from erosion of the gastric mucous membrane when associated with hyperacidity or hypersecretion.

An admixture of bile, producing a yellowish or greenish discoloration, may occur with any type of vomiting, but more especially from an empty stomach and in that associated with duodenal obstruction.¹ A biliary vomiting is frequently observed in peritonitis and may be due to the fact that there is no counterpressure from the gastric contents to prevent regurgitation from the duodenum. This green vomitus may not always be due to the presence of bile, but may come from contamination with various chlorophyll-containing organisms.

Almost all types of vomitus contain mucus. In some cases we find abundant tough, slimy masses which seem to be indicative of mucous catarrh of the stomach or of a diminution in the amount of hydrochloric acid.

¹ See Pannett and Wilson, *Brit. Jour. Exp. Path.*, 1921, II, 70; Deusch and Rüup, *Deutsch. Arch. f. klin. Med.*, 1922, CXXXVIII, 165.

Fecal vomiting is a sign either of complete motor insufficiency of the intestine as found at times in peritonitis, or indicates intestinal obstruction, either in the lower part of the small intestine or in the large bowel. The brownish-black color of this vomitus and the distinct odor render it very characteristic.

Asiatic cholera and cholera nostras are associated with a vomitus which is abundant, alkaline in reaction, contains white flakes of mucus and epithelial cells, and large numbers of bacteria, both Koch's spirillum and the Finkler-Prior spirillum and various other unidentified types. This vomitus of cholera is known as the "*rice-water*" vomitus.

The time at which vomiting occurs is frequently of great importance from the diagnostic standpoint. If it be at the height of digestion and during intense pain the condition is probably one of ulcer. If during or shortly after eating we may have either gastritis, a neurosis, or cancer. If it is frequent in the morning before breakfast and seems to be independent of eating the condition is probably one of dilatation. While these statements are not infallible, yet they are applicable in the majority of cases.

Gastric Contents After Test Meals.

The amount of material obtained after a test meal has some diagnostic importance. As previously stated, one obtains after an Ewald or Boas meal from 20 to 50 c.c. of contents, but these figures may vary to as high as 500 c.c. Hypersecretion or motor insufficiency are the chief causes of such increased amounts, the former being more probable if a large amount of free hydrochloric acid is present along with the excessive amount of fluid.¹ A larger proportion of solid undigested material is observed in cases of pure motor insufficiency, but we frequently have a combination of both conditions. Absolute proof of the diminished motility is found in the presence of more than a trace of food in the stomach seven to eight hours after a meal. The general appearances of the material obtained after a test meal are those previously discussed.

IV. MICROSCOPIC EXAMINATION

The microscopical examination of the gastric contents is usually made on material withdrawn from the stomach after test meals, but the vomitus is occasionally examined. The gastric juice is practically never free from remnants of food, such as meat threads or starch granules, although nothing has been taken for many hours. Moreover, small masses of mucus, which occasionally assume a snail-like spiral form, and saliva which is recognized by the presence of large flat epithelial cells and the so-called salivary corpuscles are quite frequent. A few bacilli and yeast cells are almost always observed.

As such elements as the ones above mentioned are present in all gastric juice, we must not attach undue importance to the presence of small amounts of such material in the contents obtained after a test meal. After the Ewald meal one rarely finds anything beyond the presence of numerous starch

¹ van Spanje (Nederl. Tijdschr. v. Geneesk., 1913, LVIII, 213) calls attention to the dry test meal of triscuit in estimating the motor efficiency of the stomach.

granules and more or less mucoid material, along with bacteria of the various types which flourish particularly in the buccal and gastric cavities.¹

In cases associated with diminished motility of the stomach we may find remains of food which has been introduced many hours previously. In such specimens we observe numerous fat globules or fatty acid crystals, many vegetable fibers and cells and a few red blood-cells which have come from slight abrasion of the mucous membrane of the pharynx by the stomach-tube. These red cells usually are much altered in appearance by the hydrochloric acid and do not show their ordinary hemoglobin color, but take on a more brownish tint, which is due to the presence of hematin.

Boas-Oppler Bacillus.

This organism is found quite commonly in patients suffering with carcinoma of the stomach, and is almost always absent in nonmalignant disease. It is found more frequently in the gastric contents at a time when lactic acid



FIG. 16.—Boas-Oppler bacilli. (Hemmeler.)

is present in large amounts, so that in the incipient stages of carcinoma these organisms may be absent. These bacilli are very long (3 to 10 microns), 1 micron broad, and are frequently joined end to end forming very long chains. They are readily stained with the usual aniline dyes and by Gram's method and, on treatment with iodine, take on a brown color which distinguishes them from the large mouth bacillus (*leptothrix buccalis*), which stains blue with iodine. This organism appears to be identical with the bacillus bulgaricus, one of the lactic acid group, and is not an organism *sui generis*.² It is not absolutely pathognomonic of carcinoma of the stomach, although Smithies³ demonstrates its presence in 93.8 per cent. of 566 cases of pathologically verified carcinoma. Owing to the absence of hydrochloric acid in such cases, this organism develops and produces the lactic acid found.

¹ See Lyon, Am. Jour. Med. Sc., 1915, CL, 402; Kopeloff, Proc. Soc. Exp. Biol. Med., 1921, XIX, 110; Inkster and Gloyne, Brit. Med. Jour., 1921, II, 1024; Van der Reis, Arch. f. Verd.-Kr., 1921, XXVII, 353; Hajós, Wiener Arch. f. inn. Med., 1922, III, 453.

² Galt and Iles, Jour. Path. and Bacteriol., 1914, XIX, 239; Heinemann and Ecker, Jour. Bacteriol., 1916, I, 435; Gorini, Jour. Bact., 1922, VII, 271.

³ Jour. Am. Med. Assn., 1913, LXI, 1793.¹

Sarcinæ.

Occasionally in normal gastric juice and especially in cases of dilatation with marked fermentation one finds the so-called *sarcinæ ventriculi* which are cocci arranged in squares or tetrahedra which resemble, very much, cotton bales. These organisms have no pathologic significance, but are indicative of stagnation of gastric contents. Along with these sarcinæ one may find large numbers of yeast cells.¹

Protozoa.

These unicellular parasites have been occasionally found in the gastric contents. Flagellates, amebas, and monads seem to be more frequent than the other types of protozoa.² They seem to be more commonly found in cases of carcinoma of the stomach.

Fragments of Tissue.

Frequently small shreds of mucous membrane are found in the expressed gastric contents. One finds these in cases of chronic gastritis, ulcer, hyperchlorhydria, and especially in cancer. These tissue fragments should be studied carefully under the microscope, as not infrequently a diagnosis of cancer is possible from such examination.

Crystals.

Various types of crystal are occasionally noted in the gastric contents, among which may be mentioned bile acids, cholesterin, fatty acids, leucin, tyrosin, and calcium oxalate. If the reaction of the juice is alkaline triple phosphate crystals may appear.

V. CHEMICAL EXAMINATION

The chemical examination of the gastric juice is the most important of all laboratory methods in the diagnosis of various pathologic gastric conditions. As previously stated, the acidity of gastric juice is referable to the presence of free and combined acids. The free acidity is traceable largely to hydrochloric acid, although organic acids, such as lactic, acetic, and butyric, may increase the free acidity under abnormal conditions. Besides this free acidity, we have hydrochloric acid which is bound chemically to the protein substances and does not react with tests for free acidity. There are also present in the gastric juice acid salts, especially the sodium dihydrogen phosphate (NaH_2PO_4).

Besides these factors which have to do with the reaction of the gastric juice, we find certain ferments which act only in the presence of the free hydrochloric acid.³ The first of these, pepsin, has the power of acting upon albumin in an acid medium and converting it, through various stages, into lower splitting products of albumin. This peptic digestion will be discussed in detail later. A second ferment, known as rennin, lab, or chymosin, has the

¹ See Anderson, Jour. Infect. Dis., 1917, XXI, 341; Gerhard, Münch. med. Wehnschr., 1919, LXVI, 1400; Burget, Jour. Bact., 1920, V, 299; Heissen, Arch. f. Verd.-Kr., 1921, XXVII, 218.

² See Smithies, Am. Jour. Med. Sc., 1912, CXLIV, 82.

³ See Ewald, Deutsch. Arch. f. klin. Med., 1912, CVI, 498; Michaelis, Deutsch. med. Wehnschr., 1920, XLVI, 126; Northrop, Jour. Gen. Physiol., 1920, II, 465; Ibid., III, 211.

power of curdling milk by coagulating the casein. A third ferment, lipase,¹ acts upon fat, especially when this is present in a finely divided form. This lipolytic action is not ordinarily great, but should nevertheless be remembered. The experiments of Sahli show that this action is negligible during the period covered by the administration of his test meal.

Although many statements have been made to the contrary, the gastric juice, through the agency of the hydrogen ions of its free hydrochloric acid, acts upon certain polysaccharides, especially cane sugar, hydrolyzing them into the simpler monosaccharides. Careful determinations indicate that the speed of inversion is about the same as that of an equal strength of hydrochloric acid, so that we do not need to assume any ferment action.

Besides these substances gastric juice contains a small amount of albumin, carbohydrates, and various inorganic salts.²

(1) Total Acidity.

As previously stated, the total acidity of the gastric juice is referable to the presence of free and combined hydrochloric acid, organic acids, and acid salts.³ This factor may be readily determined by titrating 10 c.c. of gastric juice with tenth-normal sodium hydrate, using phenolphthalein as an indicator. This indicator is colorless in the presence of acid and becomes red at the point of neutralization, being used as a 1 per cent. alcoholic solution. On adding a few drops of this solution to the unfiltered gastric contents, a white cloud will be observed due to the precipitation of the reagent by the water of the gastric juice. The titration is carried to the point at which the addition of sodium hydrate produces a definite pink color which remains permanent and does not deepen on the addition of further alkali. If sodium chlorid be added to the point of saturation of the gastric contents, the end point becomes somewhat sharper owing to the diminished dissociation which the disodium hydrogen phosphate undergoes into sodium dihydrogen phosphate in the presence of increased sodium ions. This precaution is rarely taken, however, as the clinical result is never so accurately determined as is the scientific factor.

The total acidity varies between rather wide limits. Normally it ranges from 75 to 100°, being made up of approximately 50° of free hydrochloric acid, 25° of combined hydrochloric acid, and 25° of organic acids and acid salts. The chief variation under normal conditions is an increase in the combined hydrochloric acid and a decrease in the organic acids and acid salts.

In pathologic conditions we may find the total acidity high with very little free hydrochloric acid, or we may find the total acidity low, with a normal amount of hydrochloric acid present.

Sahli has stated the variations in the acid factors of the stomach contents as follows: If the total acidity is high and the hydrochloric acid is normal, the high acidity can be due only to a deficient motility and absorption and hence we find an increase in the organic acids. Such a gastric juice may show

¹ See Hull and Keeton, *Jour. Biol. Chem.*, 1917, XXXII, 127.

² See Huber, *Am. Jour. Physiol.*, 1917, XLII, 404, as to origin of the gastric ammonium salts.

³ Bartha and Malgoyre (*Jour. Méd. de Bordeaux*, 1920, XCI, 63) show that the gastric juice may either increase or lose in acidity on standing.

lactic acid, but will more probably give the tests for the other organic acids. A low total acidity with an excess of hydrochloric acid shows that the motility and absorptive powers of the stomach are good. If the total acidity be moderate and free acid small in amount, a poor motility may be assumed. Generally speaking, when much lactic acid is present we find low HCl and only combined HCl; that is, diminished secretion and diminished motility. No lactic acid is found when the HCl is normal or increased.

(2) Free Hydrochloric Acid.

A number of tests have been devised for the detection of free hydrochloric acid in the stomach and its differentiation from lactic and acetic acids. It should be remembered that the tests outlined below are not specific tests for hydrochloric acid, but are common to all mineral acids. Many of these tests react also with the organic acids, providing they are present in sufficient concentration. The efficiency of any acid is due merely to the ionic decomposition which it suffers when in solution; in other words, is due to the presence of free hydrogen ions. Hydrochloric acid appears to be more efficient in the digestive processes than do the organic acids, owing simply to its greater degree of dissociation. The tests commonly employed in clinical work are based upon the reaction which certain coloring matters undergo when treated with free hydrochloric acid. These tests can be, therefore, only approximate and must be used with discretion in scientific work, although in clinical work they are near enough for all purposes. Were we able to express completely the stomach contents and thus to obtain material which would give us absolute data, we would then require better clinical methods. What should be measured in testing stomach contents for free acidity is the number of hydrogen ions which the gastric juice contains, since this is the important factor in the efficiency of the juice.¹ This may be determined directly by measuring the speed of any catalytic reaction due to hydrogen ions, the most convenient one being the rate of inversion of cane sugar.

It is wise to have some quick method by which one may determine the presence of a free acid in the gastric juice. This may be done with litmus which will, however, not show the presence of free hydrochloric acid to the exclusion of other acids or acid salts. To determine whether the acid reaction is due to free acid it is customary to employ the Congo-red paper. This consists simply of filter-paper which has been saturated with an alcoholic solution of Congo-red and dried. On treating gastric juice with such paper we obtain a blue color in the presence of free acid.² This paper reacts with a blue color

¹ See Michaelis, *Die Wasserstoffionen Konzentration*, 1914, Springer, Berlin; Menten (*Jour. Biol. Chem.*, 1915, XXII, 341) shows that the P_H of undiluted normal gastric juice, under varying conditions, ranged from 0.92 to 1.58.

² Holmgren (*Deutsch. med. Wchnschr.*, 1911, XXXVII, 247) has devised a quantitative, as well as qualitative, test for free HCl using the capillarity of such Congo-red paper as a basis of estimating the quantity of HCl, the discolored zone varying in width with the degree of acidity. This method of "capillary analysis" is, however, not as constant and reliable for quantitative purposes as are the titration methods, as Mattisson (*Arch. f. Verdauungskr.*, 1913, XIX, 79 and 226) and Schmidt (*Biochem. Jour.*, 1913, VII, 231) have shown. Aberg (*Hygiea*, LXXVI, 105) advises the use of tincture of cochineal or lacmoid as an indicator in this test. See, also, Sochanski, *Arch. f. Verdauungskr.*, 1941, XX, 317. Shohl (*Bull. Johns Hopk. Hosp.*, 1920, XXXI, 152) and Shohl and King (*Ibid.*, 1958) use a 0.2 per cent. solution of thymolsulphonaphthalein. See, also, Baufle, *Paris Méd.*, 1910, IX, 428; Ryffel, *Lancet*, 1921, I, 586; Lanz, *Arch. f. Verd.-Kr.*, 1921, XXVII, 282; Christiansen, *Arch. f. Verd.-Kr.*, 1921, XXVIII, 231; Silverman, *New Orleans Med. & Surg. Jour.*, 1922, LXXIV, 627.

to any free acid so that one should never assume the presence of free hydrochloric acid when he obtains a blue coloration. Although many writers state that a blue coloration is never given by free organic acids, the writer has seen too many cases in which distinct action was referable either to free lactic or free acetic acids to agree with this statement.¹ The tests which are applicable to the detection of free mineral acids in general may be used as indicative of free hydrochloric acid, as this is the only mineral acid which one would ordinarily find in the gastric contents.

Qualitative Tests.

(a) Töpfer's Test.

This test is based upon the coloration which a 0.5 per cent. alcoholic solution of dimethyl-amido-azobenzol takes when treated with gastric juice containing free hydrochloric acid. A few c.c. of unfiltered gastric juice are placed in a dish and one or two drops of the above solution added. In the presence of free mineral acids a carmin red color is obtained. This reagent is a very delicate one and does not react to organic acids unless they are present in amounts exceeding 0.5 per cent. The coloration with free hydrochloric acid varies in intensity with the amount of acid present, and may range from a deep orange to an intense carmin. According to Simon, lactic acid does not give the typical red color with this reagent, especially if albumoses are present, unless it be in a concentration of at least 1 per cent. This reagent will detect the presence of 0.02 parts of hydrochloric acid per thousand.

(b) Günzburg's Test.

The reagent employed in this test consists of 2 grams of phloroglucin and 1 gram of vanillin dissolved in 30 c.c. of absolute alcohol. This yellowish solution should be kept in dark bottles, as it gradually changes to a dark red and then to brown when exposed to the light. Boas claims that the reagent becomes more delicate and stable if one dissolves the phloroglucin and vanillin in 100 c.c. of 80 per cent. alcohol.

Two or three drops of this solution are added to an equal amount of the gastric juice contained in a porcelain dish and the mixture evaporated over a water-bath. In the presence of free mineral acid a rose-red color is developed, varying in intensity with the amount of acid present. This mixture must not be boiled or heated too rapidly as the resulting color will then be brown or brownish-red and may mislead one into believing that no free hydrochloric acid is present.

This test does not react to organic acids or to acid salts, nor is it interfered with by the presence of products of food digestion. It may, therefore, be used with the unfiltered gastric juice. This test reacts with the rose-red color in the presence of 0.05 parts of HCl per thousand.²

(c) Boas' Test.

This reagent consists of 5 grams of resorcin and 3 grams of cane sugar dissolved in 100 grams of 95 per cent. alcohol. It has the same delicacy as

¹ See Luers, *Kolloid Ztschr.*, 1920, XXVI, 15.

² See Christiansen, *Biochem. Ztschr.*, 1912, XLVI, 24, 50 and 71.

Günzburg's test and is more stable. The test is applied in the same way as the preceding, taking particular care to use a low flame in evaporating, and gives a rose-red or vermilion color in the presence of mineral acids. This color gradually fades on cooling and is not given by organic acids or acid salts.

(d) **Tropeolin Test.**

The reagent for this test is a saturated alcoholic solution of tropeolin oo. This test is applied in the same way as the preceding and gives a lilac-blue color in the presence of free acid.¹ This test is not as delicate as the preceding, reacting only in the presence of 0.3 parts of free hydrochloric acid per thousand, and has the objection that it strikes the blue color much more easily with the organic acids.

Other tests have been advocated for the qualitative detection of free hydrochloric acid, but they are not as delicate as the above and have nothing to justify their existence.² Of the tests given, the Günzburg test seems to be the most reliable, although the Töpfer's test is clinically sufficient and has the advantage of being much less expensive and more convenient than is the former reagent.

Quantitative Methods.

The quantitative estimation of free hydrochloric acid is of great importance in the study of all pathologic conditions of the stomach. Any determination made with our present methods must have reference to the fact that the acidity of the gastric juice is due almost entirely to the free hydrochloric acid. While this is not absolutely true, yet the organic acids are rarely present in sufficient amounts to react with the indicators in conditions in which hydrochloric acid is normal or increased in amount. However, in the conditions associated with diminished amount of hydrochloric acid one must be on his guard in the interpretation either of the qualitative or quantitative tests for free hydrochloric acid. The writer has seen several cases in which all of the indicators, with the exception of Günzburg's reagent, showed positive results for hydrochloric acid, the cases being those in which no free hydrochloric acid was actually present. Bearing this in mind we may determine the acidity, referable to free hydrochloric acid, by the titration of a known amount of the unfiltered gastric juice with tenth-normal sodium hydrate solution, using as indicators the solutions mentioned under the head of qualitative tests.³

Mintz Method.

As the Günzburg reagent is the most delicate and reliable of all the tests for free hydrochloric acid, it is wise to use this reagent as an indicator. The test as usually followed by the writer is to add 20 to 30 drops of the Günzburg reagent to 10 c.c. of the gastric juice. On adding the sodium hydrate solution no color change will be visible, as the reaction takes place only when the solution is warmed. This warming cannot be done directly as the evaporation would necessarily have to proceed to the point at which loss of hydrochloric

¹ Fittipaldi (Gazz. d. osp., 1913, XXXIV, 289) uses this reagent for quantitative purposes.

² See, however, Cipallina, Riforma Med., 1913, XXIX, 505.

³ See Knapp, New York Med. Jour., 1913, XCVII, 437.

acid might occur. Following the recommendation of Sahli, the rod with which the solutions are stirred is warmed before being used. As the neutralization point is reached the red color, originally noted on the sides of the warm rod, disappears.

Töpfer's Method.

This method is the simplest, is the most generally used, and at the same time is one of the most delicate of all the clinical quantitative methods for free hydrochloric acid. It consists in the use of dimethyl-amido-azobenzol as an indicator, the titration of the unfiltered gastric juice being done with tenth-normal sodium hydrate solution. Although this reagent does give, under some conditions, a red color in the absence of free hydrochloric acid when the organic acids are largely increased, yet such conditions are so rarely found in clinical work that the result of test-tube experiments cannot be applied to clinical cases. The experiments of Simon show that lactic acid must be present to the extent of 1 per cent. before any cherry-red color is obtained, providing albumoses are present. As these latter substances are always found in the gastric juice after intake of protein material, one can readily see that lactic acid need not be considered. We find, however, conditions associated with fermentative processes in the stomach in which acetic and butyric acids are present in fairly large amounts. These acids will give a red color with the indicator, but should not mislead as their strong odor in such concentrations permits of easy recognition.

In the titration the sodium hydrate solution is added from a buret to the unfiltered gastric juice to which one or two drops of indicator are added for every 10 c.c. of juice. In the presence of free hydrochloric acid this indicator strikes a distinct cherry-red tone and thus enables the worker to decide at once as to the presence or absence of the acid. No evaporation is necessary, hence the test has the advantage of simplicity and does not occasion any loss of substance. As the sodium hydrate solution is added the reddish tint of the mixture changes to a distinct yellow. The titration must be carried to the point at which every trace of red disappears and the color becomes a pure yellow. This reaction requires considerable experience, hence the writer would advise the student to make his titration with a known solution of hydrochloric acid so that he may become familiar with the end point.

Although the writer formerly used the filtered contents in his estimations, he finds that filtration causes distinct changes in the composition of the fluid, owing to adsorption of free hydrochloric acid. He advises, therefore, the use of the thoroughly mixed unfiltered contents, unless there is a very large amount of mucus present. Under this latter condition one may use the filtered contents. If one wishes to clarify the fluid for the determination of pepsin, it is wise to centrifuge the specimen instead of filtering.¹

Amount of Free Hydrochloric Acid.

Although the general idea prevails that the normal human gastric juice contains free hydrochloric acid to the extent of 0.2 to 0.3 per cent., recent

¹ See Christiansen, *Biochem. Ztschr.*, 1912, XLVI, 82; Zunz, *Handb. der Biochem. Arbeitsmeth.*, 1914, VIII, 44; Seidl, *Arch. f. Verdauungskr.*, 1915, XXI, 196.

studies, under properly controlled conditions, on both lower animals and man, indicate that this amount reaches a constant figure more nearly that of 0.5 per cent. in cases in which the freshly secreted normal gastric juice is examined. Carlson¹ calls attention to the fact that normal human gastric juice secreted at a fairly high rate is equal in total acidity to the acidity reported by clinicians for so-called hyperacidity, and states that "there is no evidence that the gastric glands under any pathologic conditions are able to or do secrete a juice of higher than normal acidity." Although it is impossible to deny the existence of cases of hyperacidity, these experiments and conclusions are, to a certain extent, in conformity with clinical observations, as we unquestionably observe many cases of high acidity with no symptoms of this condition and, on the other hand, symptoms of hyperacidity in cases of real hypoacidity. Hence, we are forced to the conclusion that the same amount of acid may indicate a hyperacidity in one case and not in another. The fallacy of relying on percentage relations instead of relating the symptoms of hyperacidity to a hypersensitiveness of the gastric mucosa toward certain degrees of hydrochloric acid is, therefore, evident.

This normal acidity does not remain in the digesting stomach at this higher level for a great length of time, being relatively soon lowered to a level of optimum acidity of about 0.2 to 0.3 per cent., according to Boldyreff,² by neutralization by the mucus secreted by the gastric glands, by the combination of the acid with the protein of the food and, especially, by reflux of alkaline juices from the duodenum into the stomach. At the height of digestion we find, therefore, that the acidity of normal gastric juice may range from 0.2 to 0.5 per cent., this optimum varying in different subjects and with the type of food in the stomach. Under ordinary conditions this excess of free hydrochloric acid should be evident in 45 to 60 minutes after an Ewald breakfast, but this time varies with the individual case. It is evident, therefore, that a diagnosis of hyperacidity or hypoacidity cannot be absolutely made by reference simply to the chemical analysis of a gastric contents.³

Certain clinical conditions lead to an excretion of gastric juice which is normal, increased, or diminished in amount of free hydrochloric acid. We must, therefore have a method of interpretation of the free HCl acidity of the stomach. It is customary to report the results of titration of a given specimen of gastric juice in one of two ways:

(1) We may represent the acidity referable to free hydrochloric acid by the number of c.c. of tenth-normal sodium hydrate necessary to neutralize 100 c.c. of unfiltered gastric juice, using dimethyl-amido-azobenzol as an indicator. This is called degree or percentage of acidity. Thus, if 5 c.c. of tenth-normal sodium hydrate were used to neutralize 10 c.c. of unfiltered gastric juice, the degree or percentage of acidity would be, obviously, 50.

¹ *Am. Jour. Physiol.*, 1915, XXXVIII, 248; also, Menten, *Jour. Biol. Chem.*, 1915, XXII, 341; Rosemann, *Virchow's Arch.*, 1921, CCXXIX, 67.

² *Quart. Jour. Exper. Physiol.*, 1914, VIII, 1.

³ See Rehfuess and Hawk, *Jour. Am. Med. Assn.*, 1914, LXIII, 2088; Yagüe, *Arch. Esp. de Enf. del ap. Dig.*, 1910, II, 528; Hayes, *New York Med. Jour.*, 1920, CXII, 5; Rehfuess and Hawk, *Am. Jour. Med. Sc.*, 1920, CLX, 428; *Jour. Am. Med. Assn.*, 1920, LXXV, 449.

(2) We may report the actual amount of free hydrochloric acid present. This is the more scientific way, as we have a much better means of comparison with the normal standards. One c.c. of tenth-normal sodium hydrate neutralizes 0.00365 gram of free HCl. If, now, we multiply this factor by the number of c.c. necessary to neutralize 100 c.c. of unfiltered gastric juice, we obtain a figure representing the absolute amount of free HCl in the gastric content. Thus, if the gastric juice showed an acidity of 50 degrees, we would have 0.1825 (50×0.00365) gram of HCl. The usual text-book statement is that the free hydrochloric acid is normally about 40 degrees, but the writer is accustomed to consider an acidity of 50 to 55° as much more nearly normal than one of 40.

Euchlorhydria.

This is a condition in which the amount of free hydrochloric acid is between 0.2 and 0.3 per cent. In speaking of percentage one must not confuse the two types of percentage reckoning. Thus, 10 per cent. or 10° of HCl represents only 0.0365 gram or true per cent. of hydrochloric acid. The lower figure of 0.2 per cent. is given for euchlorhydria owing to the fact that normal variations may permit of this low point, although the higher figure of 0.3 per cent. is the more usual. When this euchlorhydria exists in the presence of clinical symptoms pointing to gastric disturbance, we usually have to do with a neurosis. Gastritis may be absolutely ruled out, a carcinoma excluded except when the new growth has taken place upon an old ulcer and an ulcer practically always ruled out, although this latter may show an euchlorhydria. This condition may be associated with a certain amount of atony along with more or less marked dilatation.

Hypochlorhydria.

This is a condition associated with excretion of the gastric juice showing 0.1 or a lower per cent. of hydrochloric acid. It is found especially in sub-acute or chronic gastritis, in incipient carcinoma, in fevers, severe anemias, many mental diseases, passive congestion due to valvular heart lesions, many cases of chronic nephritis, and dilatation of the stomach.¹ Some rare cases of ulcer of the stomach show a low degree of free hydrochloric acid, but this is not usual.

Anachlorhydria.

This is a condition characterized by the excretion of the gastric juice showing the complete absence of free hydrochloric acid. This condition has been supposed to be pathognomonic of gastric carcinoma. There are, however, many cases of cancer which show either a hypo- or a hyperchlorhydria, and there are, also, many other conditions which show an anachlorhydria. Thus we find this condition in a large majority of cases of advanced chronic gastritis, in the severe anemias, especially of the pernicious type, in neurasthenia and hysteria, in many severe febrile diseases, and in atrophic gastritis.²

¹ Schalek (Jour. Am. Med. Assoc. 1921, LXXVI, 1005) calls attention to the possibility that hypochlorhydria may have some etiologic relation to erythema multiforme.

² Ohly (Deutsch. med. Wchnschr., 1913, XXXIX, 1402; Arch. f. Verdauungskr., 1915, XXI, 128) calls attention to the fact that 70 to 80 per cent. of all cases of progressive cholecystitis show an anacidity or actual achylia, although a slight hyperacidity may obtain in the incipient stages. This is of importance in differentiating a pyloric or duodenal ulcer from cholelithiasis. See, also, Hernando, Arch. des Mal. de l'App. digestif, 1914, VIII, 274; Grünfelder, Ztschr. f. exp. Path. u. Ther., 1914, XVI, 141.

Hyperchlorhydria.

This condition exists when we have the excretion of a gastric juice showing more than 0.2 per cent. hydrochloric acid. This figure may run anywhere from 0.2 to 0.9 per cent.¹ It is a very common occurrence in nervous individuals, in ulcer of the stomach, some cases of chlorosis, in some chronic cachexias, in the early stages of chronic gastritis, in carcinoma which is grafted on to an old ulcer, in continuous hypersecretion, in chronic passive congestion of the stomach, and in cases of migraine.²

It will thus be seen that the hydrochloric acid of the gastric juice varies under clinical conditions to quite an extent. While an increased amount of hydrochloric acid is usually present in ulcer of the stomach, we must not necessarily make our diagnosis on this point alone. Likewise in carcinoma of the stomach we should not exclude this condition if the examination of the stomach contents does not show a lessened amount of hydrochloric acid or even a total absence, as many cases of carcinoma may show all varieties of chlorhydria.

(3) Combined Hydrochloric Acid.

As the hydrochloric acid first secreted by the gastric glands combines with the protein of the food material, it is necessary to have some method by which we may determine just how much of this material has been formed in the stomach. The physiologically active hydrochloric acid consists of both the free and combined acid so that we may have only a slight amount of the free acid, but a relatively large amount of the combined. Not infrequently we find cases which show no free hydrochloric acid, but quite a percentage of the combined acid, indicating that a certain amount of acid has been secreted by the gastric juice. This combined hydrochloric acid has, therefore, a certain clinical importance and should be investigated in every case.³ No direct methods are known for its determination so that we must resort to indirect methods.

Method of Martius and Lüttke.

This method, as modified by Reissner, is much more applicable to scientific work than to clinical investigations, as it is too complicated and time-consuming for the ordinary practitioner. It is based upon the facts that the free hydrochloric acid as well as the acid combined with protein material escape upon incineration of the gastric juice, while the inorganic chlorin in combination with inorganic bases remains in the ash. If the total amount of chlorin present in the filtered gastric juice be determined (a), and then the amount of chlorin in the ash (b) investigated, subtraction of the latter (b) from the former portion (a) will give the amount of chlorin referable to free and combined hydrochloric acid except a small loss referable to volatilization of ammonium

¹ See page 76.

² Surmont and Dehon (*Arch. des Mal. de l'App. digestif*, 1914, VIII, 246) believe that defective elimination of salt by the kidneys is often compensated for by hyperchlorhydria.

³ See Grund, *Deutsch. Arch. f. klin. Med.*, 1913, CIX, 500; Shohl and King, *Bull. Johns Hopk. Hosp.*, 1920, XXXI, 162; Cohen (*Jour. Biol. Chem.*, 1920, XLI, 251) shows that the total chlorids are secreted more or less constantly regardless of the free acidity of gastric juice.

chlorid. If the gastric juice be neutralized with sodium hydrate before it is incinerated and the chlorin in this ash determined, the amount of this chlorin (a') subtracted from (a) represents the ammonium chlorid volatilized. Hence $a' - b$ equals the free and combined HCl. By now determining the amount of free hydrochloric acid according to Töpfer's method we may at once calculate the combined hydrochloric acid by subtracting this result from the amount of free and combined hydrochloric acid previously obtained. The method of determining the chlorin will be fully discussed in the section on urine to which the reader is referred.

Method of Töpfer.

This method embraces three separate determinations. In the first place the total acidity of the gastric juice is determined by titration of 10 c.c. of unfiltered gastric juice with tenth-normal sodium hydrate, using phenolphthalein as an indicator. This result is termed a . This indicator has the advantage of reacting toward anything of an acid nature, and will give us, therefore, the different factors which go to make up the total acidity of the gastric juice, namely, the free and combined acids, the organic acids, and the acid salts.

Having determined this factor (a), a second portion of 10 c.c. of gastric juice is titrated with tenth-normal sodium hydrate solution using a 1 per cent. aqueous solution of alizarin (alizarin monosulphonate of sodium) as an indicator. Two or three drops of this indicator are added to 10 c.c. of unfiltered gastric juice when the mixture becomes distinctly yellow. The titration is carried to the point of production of a pure violet color which does not deepen on the further addition of alkali. As this reaction demands the recognition of a change from yellow through a faint violet to a deep violet color the worker must have considerable practice before he is able accurately to determine the end point. The result is termed b . No trace of red should be present, a pure violet color being the true reacting point. This color may be observed by treating a few drops of alizarin solution with a 1 per cent. solution of sodium carbonate. Alizarin reacts with free acid, both mineral and organic, and with acid salts, but not with organically bound HCl. If, therefore, we subtract the figure obtained when alizarin is used as an indicator (b) from that obtained with phenolphthalein (a) the result will be the combined hydrochloric acid ($a - b$).

If now we add this combined hydrochloric acid to the free hydrochloric acid, which has been obtained by titration of the gastric juice using dimethyl-amido-azo-benzol as an indicator, (c), we obtain the total physiologically active hydrochloric acid ($c + (a - b)$). The difference between the total acidity and this factor gives us the amount of organic acid and acid salts present. ($a - (c + (a - b))$). If but a small amount of gastric juice be available for chemical examination, recourse may be had to a modification suggested by Einhorn. This is a double titration of the same portion of juice. A few c.c. (5) of unfiltered gastric juice are treated with a few drops of dimethyl-amido-azobenzol and the solution titrated for free HCl with sodium hydrate. When the point of neutralization of the free acid is reached a few drops of phenolphthalein solution are added and the titration continued to the point of neu-

tralization of total acidity. These indicators do not interfere at all with one another as their reacting points are usually widely different. The writer has found this method reliable and convenient.

Hydrochloric Acid Deficit.

In those cases in which the gastric contents show no free hydrochloric acid, it is customary to determine the HCl deficit. By this is meant the amount of hydrochloric acid which must be added to the gastric contents before it shows a reaction for free acid. This amount will naturally depend on the amount of combined HCl already present, the amount of protein in the gastric contents and the amount of alkali secreted. Sahli suggests the expression "*saturation deficit*" for this figure. Ten c.c. of unfiltered gastric juice are titrated with tenth-normal hydrochloric acid, using dimethyl-amido-azobenzol as an indicator and titrating to the point of production of the red color. The result is expressed in terms of degrees as under the representation of the free hydrochloric acid. This factor enables one to follow the course of the disease, showing how little hydrochloric acid is excreted for combination with the proteins of the food.

(4) Organic Acids.

The organic acids, outside of the lactic acid, have very little clinical significance. The food practically always contains a certain amount of fatty acids which appear in the stomach contents and contribute to the total acidity. In the normal digestion of the carbohydrates, lactic acid is practically always formed, so that excess of this acid would indicate excessive fermentative processes in the stomach, due to a combination of diminished amount of hydrochloric acid along with a lessened motility of the stomach. Other acids, such as butyric and acetic acids, are formed in this same process of carbohydrate fermentation, so that the organic acids may represent a large portion of the total acidity. Besides this, bacterial decomposition, in the absence of hydrochloric acid, plays a rôle in the production of these fatty acids. The fat-splitting ferment, lipase, may produce these organic acids in fairly large amounts.

Total Organic Acid.

It is sometimes of importance to know just how much organic acid is present in the stomach contents. This may be done directly by the Hehner-Maly method, which is based upon the fact that, if a mixture of organic and inorganic acids be neutralized and then incinerated, the organic acids will be converted into carbonates while the inorganic acids remain as neutral salts. If the alkalinity of these carbonates be then determined and this factor subtracted from the total acidity we obtain directly the mineral acids. This is possible owing to the fact that the degree of alkalinity of the carbonates is equal, in terms of tenth-normal solutions, to the acidity referable to the organic acids. The technic is as follows: The total acidity of 10 c.c. of gastric juice is determined by titration with tenth-normal sodium hydrate solution as previously described. The neutralized solution is evaporated to

dryness in a platinum dish and is then incinerated. The ash is dissolved in distilled water and the alkaline solution titrated with tenth-normal oxalic acid solution. As 1 c.c. of the tenth-normal oxalic acid solution is equivalent to 1 c.c. of tenth-normal sodium hydrate solution, we subtract the factor obtained in the latter titration from that of the former and obtain directly the degree of acidity due to mineral acids. The number of c.c. of tenth-normal oxalic acid used represents directly the total organic acids present.

In this method the acid salts are included in the factor referable to mineral acids, so that we may subtract from this factor the degree of acidity, attributable to free hydrochloric acid, and obtain the amount of acid salts present. In some cases fatty acids are present which are not soluble in water and consequently are not neutralized by the addition of the sodium hydrate solution. These acids may be extracted from the neutralized solution with ether and may then be neutralized and added to the neutral aqueous solution. The mixture is now evaporated as before and incinerated. This estimation of the higher fatty acids requires the use of the unfiltered gastric contents. However, such acids do not play a large clinical rôle and may be ordinarily omitted.

(a) **Lactic Acid.**

The ordinary foods such as milk, bread, and meat contain a certain amount of lactic acid, so that any test for the presence of lactic acid can be of value only when the meal contains very little of such foods or when the portion taken in with the food has disappeared from the stomach. After the Boas meal there is always less lactic acid than after the Riegel meal, so that the former is much preferable when a special test is to be made for the presence of lactic acid. Boas has shown that under physiological conditions no appreciable amount of lactic acid is formed during the process of digestion. At the height of digestion practically no lactic acid is demonstrable in the stomach contents. This may be due to its absorption, on the one hand, or, on the other, to the fact that the hydrochloric acid interferes with the delicacy of the reactions. Pathologically, lactic acid is found in any condition associated with stagnation of the gastric contents as a result of motor insufficiency, provided the amount of hydrochloric acid is below the normal amount. As this condition of affairs is found most frequently in cases of carcinoma of the stomach, an excess of lactic acid is very strongly suggestive of malignancy, although it must be remembered that such an excess may appear in cases of benign stenosis and gastric insufficiency.¹ If the stomach be washed out the evening before giving a test meal, preferably the Boas meal, and lactic acid be found in appreciable amounts, carcinoma is the probable diagnosis. This finding of increased lactic acid and diminished hydrochloric acid is not always observed in every case of carcinoma of the stomach. In some cases periods of increased production of hydrochloric acid alternate with increased formation of lactic acid, and in some cases, especially those in which the carcinoma has developed upon the base of an old ulcer, no lactic acid may be present, but hydrochloric acid may be found in large amounts.

¹ See Rodella, *Cor.-Bl. f. Schweiz. Aerzte*, 1918, XLVIII, 1210; *Ibid.*, 1919, XLIX, 1623; Barro, *Siglo Méd.*, 1922, LXIX, 281.

Uffelmann's Test.

This test is, perhaps, more commonly employed for the detection of lactic acid than is any other, but the writer prefers the Kelling test. Uffelmann's reagent consists of 20 c.c. of 1 per cent. carbolic acid solution, to which are added one drop of dilute ferric chlorid solution and sufficient water to form a transparent amethyst-blue solution. This solution is not permanent and must, therefore, be made fresh before each test. If a few drops of the filtered gastric juice be added to 5 c.c. of this reagent, the solution will be decolorized in the presence of lactic acid, taking on a beautiful canary-yellow or greenish-yellow tint. The mere decolorization of this solution is not sufficient for a positive test. A pure lemon yellow or canary color must be present before one may assume the presence of lactic acid. Even when this color appears one must eliminate such factors as the acid sodium phosphate, cane sugar, glucose, alcohol, and various organic acids, such as tartaric, citric, or oxalic, before he can say that lactic acid is present. A considerable excess of hydrochloric acid in the gastric juice may prevent the appearance of this color and likewise a yellowish tint of the stomach contents may obscure the result. Under such conditions it is necessary to extract the gastric contents with ether, which takes up the lactic acid. The ethereal solution is then evaporated, the residue taken up with distilled water, and the test applied to this solution.¹

Kelling's Test.

This test is in reality a modification of the previous one and consists in the addition of a few drops of filtered gastric juice to a very dilute solution of ferric chlorid. As used in the writer's laboratory, the method is as follows: To a test-tube full of water are added one or at most two drops of a 10 per cent. solution of ferric chlorid. The mixture is thoroughly shaken and divided into two portions, one of which serves as a control. On now adding a few drops of filtered gastric juice to one of these portions a distinct canary-yellow color will appear in the presence of lactic acid. The color of the two solutions should be compared so that any change in the one, to which gastric juice was added, may be observed. This test has the same objections as the Uffelmann test, so that it is always wise to extract the gastric juice with ether.

Strauss' Method.

This method is, perhaps, the very best clinical method at our disposal, as it shows lactic acid when present in pathological amounts. It does not, however, give a quantitative result, nor does one seem necessary in the ordinary clinical work. Into a special separatory funnel (see cut) are introduced 5 c.c. of the gastric juice. The funnel is then filled to the 25 c.c. mark with alcohol-free ether and well shaken. The ethereal layer will take up the lactic acid from the gastric contents. After the fluids have settled the gastric juice and ether are allowed to run out to the mark 5 by opening the stop-cock, after which distilled water is added to make up the 25 c.c. volume. Two drops of 10 per cent. ferric chlorid solution are then added with a medicine dropper

¹ Gérard and Regnault, C. R. Soc. biol., 1918, LXXXI, 388, call attention to the possibility of errors arising from the presence of lactic acid in the bread of the test meal.

and the mixture well shaken. The water will now extract the lactic acid from the ether. The aqueous layer is colored an intense greenish-yellow if more than 0.1 per cent. of lactic acid is present, while smaller amounts will show a slight greenish tinge. This test may be negative if the lactic acid present is completely combined with the proteins of the gastric juice. In such cases hydrochloric acid may be added to liberate this lactic acid before shaking out with ether.

Other qualitative tests as well as several quantitative tests have been given for lactic acid.¹ Quantitative determinations of lactic acid do not seem to be of any great clinical importance, as any marked reaction for this substance is indicative of a pathologic condition whose extent may bear no relation whatever to the amount of lactic acid present. A general idea of the amount of lactic acid may be obtained by evaporating 10 c.c. of gastric juice, acidulated with a few drops of sulphuric acid, to the consistency of a syrup and then extracting this residue several times with acid-free and alcohol-free ether. The ether may be removed by evaporation and the residue taken up with water. This watery solution may now be titrated with tenth-normal sodium hydrate, each c.c. of alkali used representing 0.009 gram of lactic acid.

The method of Boas, while very exact, is much too complicated and time-consuming for clinical work so that the writer will refer to other books for a description of this test. The principle of the method is based upon the fact that when the lactic acid is heated with a strong oxidizing agent it is decomposed into acetic aldehyd and formic acid. If now the aldehyd be distilled off and transformed into iodoform by the addition of alkaline iodine solution, this iodoform may be quantitatively determined.

(b) Butyric Acid.

This acid does not occur in the gastric contents, under physiological conditions, unless much milk or carbohydrate food has been introduced. Flügge has shown that butyric acid may be derived from lactic acid and consequently may be present under the same conditions in which lactic acid is found. As butyric acid may be introduced from without and may have been formed in the mouth, one should be careful in drawing conclusions as to the clinical significance of butyric acid.

If present in any large amount, butyric acid may be usually recognized by its distinct odor which is that of rancid butter. This test may not be sufficient for the recognition of butyric acid so that it is advisable to shake out the gastric juice with ether, evaporate, and take up with water as described under Lactic Acid. If a small pinch of powdered calcium chlorid be added to

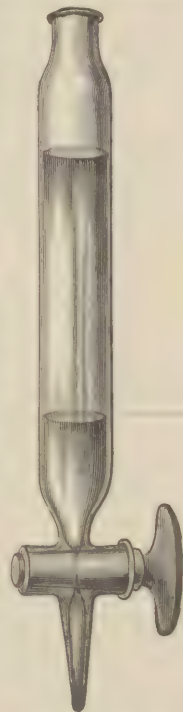


FIG. 17.—Strauss separatory funnel. (Hemmeler.)

¹ See Pittarelli, Bull. Acad. Méd., 1920, LXXXIV, 132; Hartwig and Saar, Chem. Ztg. 1921, XLV, 322.

this watery solution and the mixture warmed, butyric acid will separate from the fluid in the form of small fat drops which float on the surface and have a characteristic odor of rancid butter.

If a portion of the dried ethereal extract of the gastric juice be treated with a few drops of concentrated sulphuric acid and a little alcohol, the odor of ethylbutyrate is perceptible on slight warming. This odor is that of pineapples and is very easily recognized. This test is known as the pineapple test.

(c) **Acetic Acid.**

It is not an infrequent occurrence to find acetic acid in rather large amounts in pathological conditions. The pathologic acetic acid is formed by the bacterial decomposition of the alcohol which is produced by the action of yeast upon carbohydrates. As yeast fungi are so frequently present in cases of dilatation of the stomach, associated with stagnation of its contents, acetic acid may be found under such conditions and constitute a portion of the total acidity.

In testing for acetic acid the aqueous extract of the ethereal residue of the gastric juice is carefully neutralized with sodium carbonate solution. If a few drops of ferric chlorid solution be added to this neutralized solution a deep red color will appear if acetic acid be present. If this solution be boiled a reddish precipitate of basic ferric acetate is formed. This neutralization of the aqueous solution is an essential point in this test, as the presence of free acid will prevent the appearance of any precipitate and the presence of free alkali will cause the formation of ferric hydroxid which will mislead, as the coloration is very much the same. The writer has seen several cases in which acetic acid was mistaken for lactic acid when the Kelling test was applied. On adding gastric juice containing a large amount of acetic acid to the dilute ferric chlorid solution a change in color is observed, but in no case do you get the distinct canary-yellow color which can be possibly referable to acetic acid.

(5) **Gastric Ferments.**

(a) **Pepsin.**

The enzyme pepsin is the most important of the ferments occurring in the gastric juice. As previously stated this ferment is excreted in the form of the zymogen (pepsinogen or propepsin) by the chief cells of the fundus glands. It becomes active, that is, converted into pepsin, by the free hydrochloric acid of the gastric juice. This ferment acts only in acid media and is destroyed by the presence of minute traces of alkali.¹ Its action is continuous, a small portion being capable of digesting large amounts of albumin, providing the products of this digestion are gradually removed. Should the products of ferment activity remain in the stomach an undue length of time, this ferment will cease to be active owing to the accumulation of the products of its own

¹ Hamburger (Arch. Int. Med., 1915, XVI, 356) confirms the fact that sodium chlorid inhibits the action of pepsin as formerly suggested by Schütz and Levites. See Hamburger and Halpern, Arch. Int. Med., 1916, XVIII, 228; Ringer, Kolloid. Ztschr., 1916, XIX, 253; Biedermann, Ferment-forsch., 1917, II, 1; Davis and Merker, Jour. Am. Chem. Soc., 1919, XLI, 221. Michaelis and Rothslein, Biochem. Ztschr., 1920, CV, 60. See, also, Edie (Biochem. Jour. 1914, VIII, 193) for a discussion of the reciprocal action of pepsin and stryptin.

activity. Pepsin acts in the presence of many other acids, but the concentration of these acids must be higher than in the case of hydrochloric acid. Thus, a 0.2 to a 0.4 per cent. hydrochloric acid gives the best results with pepsin, while a 1 to a 1.018 per cent. lactic acid is necessary to bring about good results.

Very little data exists as to the amount of pepsin or of its zymogen so that we are forced to draw our conclusion regarding a normal or abnormal amount of this ferment from the rate at which known amounts of albuminous material are digested. Pepsin acts only upon protein substances, giving rise to a series of decomposition products which will be discussed later. Normally 25 c.c. of gastric juice will dissolve (digest) 0.05 to 0.06 gram of serum albumin in one hour, the same amount of coagulated egg-albumin in three hours, and a similar amount of fibrin in one and one-half hours.

A diminution in the amount of pepsin must be referable to a direct disease of the secreting gland, as general abnormalities do not affect this function as much as they do the production of free hydrochloric acid.¹ Pepsin is usually present when the free hydrochloric acid is either increased or diminished, but in cases of carcinoma, atrophic gastritis, and in occasional cases of pernicious anemia we may find no pepsin and no hydrochloric acid. Such a condition is known as achylia gastrica and occurs sometimes as a direct pathologic condition without a known etiology. It frequently happens that pepsin is present when no hydrochloric acid is found.

Qualitative Tests.

The digestive power of the filtered gastric contents will depend, of course, upon the amount of pepsin and the amount of free acid present. Artificial digestion experiments are at present the only methods by which we may test the amount of pepsin. The substances used in these digestion experiments are egg-albumin and fibrin. The fibrin may be prepared by beating freshly drawn ox-blood with a glass rod until the coagula are distinctly formed. These stringy masses are washed thoroughly in water to remove the coloring matter of the blood, are then cut into small pieces of uniform size and are kept in alcohol for a few days. These hardened masses are placed for one to two days in a neutral concentrated solution of carmin. They are then washed in water, thoroughly pressed, and are preserved in glycerin to which a little carmin has been added. Before being used they should be washed in water to remove the glycerin and free coloring matter. The egg-albumin is prepared for these experiments by boiling an egg until the albumin is distinctly coagulated. This material is then cut into cylinders of about 5 mm. in diameter with a cork borer and are then sectioned into disks 1 mm. thick. These disks may be preserved in glycerin.

In testing for the presence of pepsin 25 c.c. of gastric juice, which must contain free hydrochloric acid, are placed in a flask which contains a few pieces of fibrin or a disk of coagulated egg albumin. This flask is then placed in the incubator from 37 to 40° and allowed to remain until the protein is completely dissolved. If pepsin is present the fibrin will show signs of digestion by swell-

¹ See Carlson, *Am. Jour. Physiol.*, 1915, XXXVIII, 248.

ing up in from 15 to 30 minutes, the egg-albumin in from one-half to one hour. Within an hour and a half the fibrin should be practically dissolved, while the egg-albumin will require about three hours.

If no hydrochloric acid is present in the gastric juice a few drops of 10 per cent. hydrochloric acid are added to 25 c.c. of gastric juice and the test performed in the same manner. A positive result will indicate the presence of the zymogen, pepsinogen.

Quantitative Examination.

Certain laws have been discovered regarding the action of ferments in general and these are applicable to pepsin. Schütz has found that the relative quantities of pepsin in digesting mixtures containing the same quantity of hydrochloric acid are proportional to the squares of the quantities of albumin digested in the same time, or, in other words, the activity of a ferment varies as the square root of its amount. Nirenstein and Schiff¹ have found that this law applies only for the less concentrated pepsin solutions. If the quantity of pepsin in the digesting fluid is so large that more than 3.6 mm. of albumin (see Mett's Test) are digested in 24 hours, the above law does not give reliable values for the quantity of pepsin. The gastric juice under such circumstances must be diluted before this law applies. Sahli rightly calls attention to an important reason for diluting the gastric juice before testing for pepsin, namely, the constant presence of the products of peptic digestion which inhibit further peptic activity. The gastric juices which contain a diminished amount of hydrochloric acid are the richest in these inhibiting substances and should be carefully studied. The presence of these substances gives rise to conditions which make it impossible to arrive at accurate conclusions if the pepsin value is calculated from the pure gastric juice.

Hammerschlag's Method.

Ten c.c. of a 1 per cent. filtered solution of egg-albumin in 0.4 per cent. HCl are poured into two test-tubes. As fresh egg-albumin contains about 13 per cent. of dry protein, it should be diluted about 13 times to make a 1 per cent. solution. To one of the test-tubes 5 c.c. of gastric juice are added, to the other 5 c.c. of distilled water, both being placed in the incubator at body temperature for one hour. At the end of this time the albumin in each tube is estimated by Esbach's method (see Urine). The difference between the precipitate of albumin in the two tubes is equal to the amount of albumin which has been digested and forms, therefore, a measure of the peptic activity of the gastric juice, the square root of the amount of pepsin being proportionate to the quantity of albumin dissolved. This test is open to the objection that the albumin of the gastric juice, as well as the albumoses are precipitated by the reagent. In one hour not all of the egg-albumin will be digested, normally only about 90 per cent.

Mett's Method.

This method is, perhaps, the one most frequently used and the one which gives quite as accurate results as the others advised.² The whites of several

¹ Arch. f. Verdauungskr., 1903, VIII, 559.

² See Christiansen, Biochem. Ztschr., 1912, XLVI, 257; also, Waldschmidt, Arch. f. d. ges. Physiol., 1912 CXLIII, 180; Hernando and Alday, Siglo Medico, 1917, LXIV, 114 and 130.

eggs are mixed, in order to avoid accidental variations in the egg-albumin, and are filtered. The gas should be removed from this material by the use of a suction pump as far as possible. A number of glass capillary tubes, each from 10 to 30 cm. in length and 1 to 2 mm. in diameter, are then filled by suction with this albumin. They are then laid in the bottom of a vessel which is placed for five minutes in boiling water in order to coagulate the albumin. The tubes are taken out, wiped carefully, and the ends sealed with paraffin or sealing wax. It occasionally happens that these tubes contain air-bubbles, which will, however, disappear in a few days. These longer tubes may be kept in stock for a considerable period of time. In performing the test for pepsin by the use of these tubes of coagulated albumin, the longer tubes are cut into lengths of about 2 cm. and are placed in a small dish or watch-glass with 5 c.c. of the gastric juice, which must necessarily be acid in reaction. These dishes containing the gastric juice and filled glass tubes are then placed in the incubator for 10 to 24 hours. At the end of this time the length of the digested column at each end of each tube is measured and the average length of the column of albumin digested estimated. The square of this digestion length is the measure of the relative amount of pepsin in the gastric juice. The unit upon which one may base comparative results of the relative amount of pepsin is that quantity of pepsin by which 1 mm. of albumin in a Mett's tube will be digested in 24 hours with an acidity of 0.18 per cent. free HCl. The length which theoretically pure pepsin would give is 4 mm., the units represented by the pepsin of the gastric juice being anywhere from 0 to 256.

Nirenstein and Schiff, for the reasons previously mentioned, advise the dilution of a gastric juice before applying this test, believing that a dilution of 16 will give more nearly exact quantitative results. Their method is as follows: One c.c. of the filtered gastric juice is diluted with 16 c.c. of 0.18 per cent. HCl. The procedure is then the same as in Mett's method, the results being multiplied, of course, by 16 in order to obtain the actual number of units in the gastric juice. In some cases it has been found that a dilution of 1 to 32 gives better results, but this is rather unusual. The results of these workers show that striking differences exist in individual gastric secretions, figures ranging between 0 and 256 units being obtained. This points to the fact that the pepsin concentration is independent of the amount of acid in the gastric juice. The quantitative estimation of pepsinogen may be carried out by either one of the methods previously outlined for pepsin by rendering the gastric juice acid with hydrochloric acid up to 1 to 2 parts per thousand.

Method of Thomas and Weber.

This method¹ is based upon the digestion of an acid casein solution in 0.2 per cent. hydrochloric acid by the gastric juice. The acid casein solution is prepared by dissolving 100 grams of finely powdered dry casein in 1,900 grams of a solution of hydrochloric acid containing 5.04 grams HCl. Five c.c. of gastric juice are added to 100 grams of the casein solution. Dilute with distilled water to 250 c.c. and place in the incubator. After the end of one hour the digestion mixture is poured into 100 c.c. of 20 per cent. sodium sulphate

¹ *Centralbl. f. Stoffwechsel. u. Verdauungskr.*, 1901, II, 365.

solution, in which the non-digested casein is completely precipitated. This is collected on a weighed filter and washed with distilled water until no trace of sulphate reaction is evident. It is then dried with alcohol and ether and weighed. The difference in weight between this undigested residue and that contained in the original amount taken gives the amount of casein digested.

This method, as modified by Volhard, consists in the titration of the acidity of the filtrate from the solution to which the sodium sulphate has been added. The total acidity is higher, the more the casein is in the uncoagulated form and the increase in the acidity will vary as the square root of the amount of pepsin.¹

(b) Chymosin (Rennin).

The normal gastric juice contains a second ferment, chymosin, which has the function of coagulating milk independently of the presence of acid. The zymogen of this ferment becomes active, however, only in the presence of acids; that is, the zymogen is converted into rennin by acids. In this process of coagulating milk, insoluble casein is formed from the caseinogen of the milk by the combined action of the rennin ferment and calcium salts, while the curdling of milk is due to the precipitation of unchanged caseinogen by acids. It is evident, therefore, that this process resembles very closely that of the coagulation of the blood, as calcium salts are absolutely essential for its success. Whether rennin is identical with pepsin, as some believe, is still an unsettled question.²

Leo's Method.

Three to five drops of gastric juice are added to 5 or 10 c.c. of fresh uncooked neutral or amphoteric milk and the mixture placed in the incubator for 15 to 20 minutes. If rennin is present in normal amounts coagulation will be observed. In this process one may not be sure as to whether the curdling is due to the action of rennin or to that of the acid. Rennin action occurs typically only when no change in the reaction of the milk has taken place.

Riegel's Method.

Three to 5 c.c. of neutralized gastric juice are added to 5 or 10 c.c. of fresh milk. This mixture is placed in the incubator and left for 15 minutes, when distinct coagulation will occur in the presence of rennin. If the milk be boiled previous to treatment the result is not so typical.

Quantitative methods for the determination of rennin are at present uncertain and are even of doubtful utility. So little is known about the variations in the rennin ferment of the gastric juice that an exact determination would add little to the clinical history. According to Glässner, pepsin and rennin are both diminished in cases of tumor of the fundus, while pepsin is diminished and rennin normal in tumors of the pylorus.

¹ For other methods see Jacoby and Solms, *Ztschr. f. klin. Med.*, 1907, LXIV, 159; Fuld and Levison, *Biochem. Ztschr.*, 1907, VI, 473; Hata, *Ibid.*, 1909, XXIII, 179; Neilson and Bonnot, *Arch. Int. Med.*, 1913, XI, 395; Gesellschaft, *Ztschr. f. physiol. Chem.*, 1915, XCIV, 205; Spencer, *Jour. Biol. Chem.*, 1915, XXI, 105; Michaelis, *Deutsche med. Wchnschr.*, 1918, XLIV, 685; Farrington, Lewis and Brown, *Jour. Lab. and Clin. Med.*, 1919, IV, 635; Northrop, *Jour. Gen. Physiol.*, 1919, II, 113. Takata, *Tohoku Jour. Exp. Med.*, 1921, II, 127; Brewster, *Jour. Biol. Chem.*, 1921, XLVI, 119; Jarno, *Arch. f. Verd.-Kr.*, 1922, XXIX, 164.

² See Fuld, *Internat. Beitr. z. Path. u. Therap. d. Ernährungsstor.*, 1913, V, 104; Edie, *Biochem. Jour.*, 1921, XV, 507.

(c) Lipase.

While it is understood that lipase occurs in the gastric secretion, its action is very slight.¹ Normally, gastric digestion is not much concerned with the splitting of fat into lower products, but, as Volhard has shown, this action does occur. In testing for the presence of lipase in gastric contents it is necessary that the examination be made after the stomach is thoroughly washed out following the administration of a test meal free from fat.

This ferment may be detected qualitatively by adding a small piece of fresh neutral butter to the gastric juice and placing the mixture in the incubator for one hour. At the end of this time a distinct odor of butyric acid will be observed.

It may be said that owing to this lipolytic action statements have arisen that the Sahli test meal gives erroneous results. Careful work by Seiler shows that the amount of fat decomposed under the conditions of the test meal is so slight that it may be neglected. Volhard found that after two hours from 30 to 36 per cent. of the fat was split up into fatty acids, which aid in dissolving the bile and in forming an emulsion with the neutral fat in the intestinal canal.

The Products of Protein Digestion.

It is generally stated that the action of the pepsin and hydrochloric acid of the gastric juice upon protein material passes through the following stages: The albumin is first converted into acid-albumin (syntonin), then into albumoses, of which there are four (prot-albumose, hetero-albumose, dys-albumose, and deuto-albumose), and ultimately into peptone. It is well established that pepsin itself will not carry the hydrolysis beyond the stage of peptone or somewhat lower polypeptids. The finding of amino-acids and hexon bases, by the author as well as by others, in the contents of a dilated stomach must be interpreted as meaning a conversion of the pepsin products into lower cleavage units through the action of some other ferment, whether derived from the intestinal canal or excreted into the stomach from a malignant growth. Pepsin has, therefore, a function distinct from that of trypsin, attacking different linkings within the protein molecule. It is practically never necessary to test the stomach contents for such material in clinical work, so that the writer will refer to text-books on physiologic chemistry.

The Products of Carbohydrate Digestion.

The pure gastric juice, owing to its acid content, inverts sugars to a certain extent. The amount of this inversion depends on the number of free hydrogen ions arising from the acid, and is only very slight under normal conditions.

As previously described, the saliva converts starch into soluble substances through the stage of soluble starch, erythrodextrin, achroödextrin and finally maltose. The action of the ptyalin of the saliva is inhibited by the free acid of the gastric juice, but the action of this salivary ferment is so rapid that from 50 to 75 per cent. of the starch is converted into a soluble form.² While these products are not ordinarily tested for in the gastric contents, the fact of their formation and presence must be remembered. An excess of starchy

¹ See Takata, *Tohoku Jour. Exp. Med.*, 1921, II, 209.

² See Maxwell, *Biochem. Jour.*, 1915, IX, 323.

material in the food will lead to an increased amount of such decomposition products in the stomach when the acid of the gastric juice is not present in sufficient amount to inhibit the action of the ptyalin of the saliva.

Blood.

Blood is not a normal constituent of the gastric contents, but is found frequently in conditions associated with erosion, in ulcer, and in carcinoma. The appearance of the blood in cases of ulcer is usually that of fresh bright red blood, which may, however, be changed to a brownish substance due to the action of the excess of acid commonly present in this condition. In carcinoma the blood is more intimately mixed with the stomach contents and appears in the form of brownish-black clumps, constituting the so-called "coffee-ground" material. The tests for the presence of blood will be given under *Feces*.¹

Gases.

The stomach usually contains a certain amount of gas which may have been swallowed, may have passed into the stomach from the duodenum, or may have been produced in the stomach by processes of fermentation. The examination for these gases is not of great clinical importance, but a general idea of the different kinds of gases present seems essential. During the processes of normal digestion, nitrogen, oxygen, and carbon dioxide may occur from the protein digestion, while hydrogen, marsh-gas, and olefiant gas may arise from the carbohydrate hydrolysis. In abnormal processes of digestion we may find ammonia and hydrogen sulphid arising from decomposing protein material. The work of Boas seems to indicate that the hydrogen sulphid is more commonly present in cases of benign gastric dilatation and is rare in carcinoma. This hydrogen sulphid does not seem to be produced either in the presence of free hydrochloric or of lactic acid. The presence of hydrogen sulphid can, however, not be considered as a specific substance in the stomach contents, as Dauber has shown that almost every stomach contains bacteria which may produce this gas from sulphur-containing bodies. In cases of dilatation of the stomach, providing the motility be sufficiently diminished, we find fermentation with resulting gas production even though hydrochloric acid be present. Such a condition never occurs if the motility is normal, utterly regardless of the amount of hydrochloric acid, as in cases of diminished hydrochloric acid lactic acid will usually prevent such a process.

One may show the presence of the gases in the stomach contents by filling a fermentation tube with the well-mixed unfiltered gastric contents and placing it in the incubator for some time. If there is no gas within 24 hours it may be wise to wait at least 48 hours to permit of the proper diffusion of the gas. If gas is formed its nature may be determined by the ordinary chemical tests. This test has some value in determining the degree of stagnation of the stomach contents, but it must be remembered that a small amount of gas is contained in the normal stomach.

Other substances, such as acetone, have been found in the gastric contents in pathologic conditions, but tests for these substances are rarely of impor-

¹ See Leviton, *Jour. Lab. and Clin. Med.*, 1916, I, 761; Rutz (*New York Med. Jour.*, 1920, CXII, 619) calls attention to the futility of examining the filtrate of gastric contents for occult blood.

tance. In conditions associated with the presence of acetone in the stomach contents this substance is usually detected in the breath.

Functions of the Stomach and its Contents.

The stomach is to be regarded as a specialized portion of the alimentary tube in which the first stages of digestion of protein material take place. This occurs under the combined action of the hydrochloric acid and pepsin, the resulting products being gradually passed into the duodenum through the pylorus. An increased acidity of the gastric juice may be associated with a distinct spasm of the pylorus so that food material cannot pass into the duodenum. On the other hand, a lessened degree of acidity is associated with hypermotility, the contents passing rapidly into the intestine, where it is acted upon by the pancreatic ferments. Beside the function of digestion, principally of the protein foods, the stomach serves as a reservoir to hold the food material, allowing it to pass only in small portions into the bowel at any one time.¹ Owing to the presence of hydrochloric acid, the gastric juice is antiseptic, rendering inert many but not all types of bacteria.² Moreover, the hydrochloric acid activates the zymogens and thus permits of action upon all types of food material. The work of Pawlow has shown that the acid of the gastric juice is one of the most powerful stimulators of pancreatic secretion. The mechanism of correlation between the stomach and bowel is more easily understood if this point be borne in mind. The acid chyme coming from the stomach is poured out only in small portions at a time so that the pancreatic juice secreted may act upon the smaller portions as they are passed into the bowel.

VI. MOTILITY OF THE STOMACH

It is probably true that disturbances in the motility of the stomach are in reality of more importance than are those in the secretory activities.³ Under normal conditions of motility the food material passes into the intestine and is digested there, although no previous gastric digestion has taken place. If the motility be much impaired, stagnation of food with resulting dilatation of the stomach will occur, which will give rise to more or less serious disturbance. The motor disturbances are of three types, (1) vomiting, (2) hypermotility, and (3) motor insufficiency. The most important of these is the latter, as the former has little influence upon actual digestion in the stomach, although the patient may suffer for want of adequate nutrition; while in hypermotility the gastric disturbance will not be much noticed, owing to the fact that the food is rushed into the duodenum where it is digested. The consequences of motor insufficiency may be either disorders of secretion, decomposition, or both. Simple pathologic conditions which hinder the emptying of the stomach, such as ulcers, cicatrices, spasm of the pylorus, and simple atony, are

¹ Tangl and Erdélyi (*Biochem. Ztschr.*, 1911, XXXIV, 94) and Fejér (*Ibid.*, 1913, LIII, 168) have shown that the fats are discharged into the bowel at different rates depending upon their melting point and their viscosity. The higher the melting point the longer a fat remains in the stomach, while the more viscid a fat the less does it retard the discharge of non-fat foods.

² See Gregersen, *Centralbl. f. Bakteriöl.*, 1916, LXXVII, 353; Scheer, *Arch. f. Hyg.*, 1919, LXXXVIII, 130.

³ See Carlson, *Am. Jour. Physiol.*, 1912, XXXI, 151; Lechler, *Arch. f. Verd.-Kr.*, 1919, XXV, 69; Rehfuess and Hawk, *Jour. Am. Med. Assoc.*, 1921, LXXVI, 371.

usually associated with hyperacidity, while malignant conditions usually show a diminished secretion.¹ It is quite rare to find a case of motor insufficiency without disorder of secretion, rarer at all events than disorders of secretion without motor disturbances (Schmidt). Motor insufficiency is quite commonly followed by decomposition of the gastric contents and may even be considered the chief cause of such decomposition. Under these conditions of disturbed secretory and digestive activity associated with motor insufficiency we find the absorptive power of the stomach very much affected.

Hypermotility is seen in many cases of hyperacidity, but it must be remembered that primary hyperacidity may cause spasm of the pylorus and hence bring on a distinct motor insufficiency. We cannot, therefore, judge of the motility of the stomach from the degree of acidity of the gastric contents. An enlarged stomach is not necessarily associated with motor insufficiency. Cases of megalogastria are more or less frequent in which the motility is practically normal. When a dilatation is associated with motor insufficiency it is clinically styled an ectasia or ectasis, being known as atonic gastric ectasis if the condition is due to weakness of the muscle, while it is styled hypertonic gastric ectasis if due to pyloric stenosis. Normally, no food should be found in the stomach within seven to eight hours after taking, no matter how large the meal.²

Leube's Method.

Leube administers a Riegel test meal and washes out the stomach with a liter of water six hours after. If only very slight traces of food are found in the washings the motor power is regarded as normal.

Boas' Method.

Boas administers a simple evening meal consisting of meat, bread and butter, and tea, washing out the stomach the following morning. If any food material is found the motor insufficiency is considerable. If the stomach be washed out previous to the administration of the evening meal no food should be found in the stomach in the morning.

Method of Ewald and Sievers.

This test is based upon the observation that salol is decomposed into carbolic and salicylic acids only in an alkaline medium. As the salicylic acid is eliminated in the urine in the form of salicyluric acid, it is possible to determine the rate of passage of salol from the stomach to the small intestine. It seems necessary to state that the assumptions on which this test are based are partially wrong. In the first place salol is split into its constituents by gastric juice within 15 minutes, although the degree of dissociation is slight. Moreover, a certain amount of absorption of salicylic acid takes place from the stomach so that a reaction may be obtained in the urine within 15 minutes in cases in which no hypermotility exists.

One gram of salol is given to the patient immediately after a meal. The urine is then collected every 15 minutes for two hours and tested by the addi-

¹ See Hamburger and Friedman, *Arch. Int. Med.*, 1914, XIV, 722.

² See Faulhaber, *Berl. klin. Wehnschr.*, 1914, LI, 1355; Levy and Kantor, *Arch. Int. Med.*, 1916, XVII, 476.

tion of a small amount of ferric chlorid solution, which will give a violet color in the presence of salicyluric acid.¹

Under normal conditions, according to Ewald, a positive reaction occurs in from 45 to 75 minutes. A further delay above 75 minutes is indicative of motor insufficiency, the degree of insufficiency bearing some relation to the time of appearance of this reaction. Should no result be obtained after 24 hours a stenosis of the pylorus is highly probable.

As the writer has so frequently found a reaction for salicyluric acid in the urine within 15 minutes, which is due not to the action of the hydrochloric acid or the ferments of the gastric juice, but to the moisture, temperature, and bacteria, he is accustomed to use the time at which a reaction for this substance disappears from the urine rather than the time at which it makes its first appearance, as the basis of judgment regarding the motility of the stomach. Normally, no reaction for salicyluric acid should occur in the urine after 24 hours although Huber states that it may take 26 to 27 and hence limits his time to these latter figures. It might be wise to follow the suggestion of Sahli and determine both the time of appearance and disappearance.

Sahli has called attention to the fact that we are not justified in assuming a pyloric stenosis in case food material is found in the stomach even several days after being taken. He adds that the emptying of the stomach is regulated by the intestine rather than by the stomach itself, since nutritive substances reaching the intestine effect a reflex closure of the pylorus (von Mering's reflex) until the intestine has completed its work. The motor activity of the stomach should, therefore, be examined under conditions in which this reflex does not occur. This he determines by estimating the length of time required by the stomach to empty itself of a half-liter of water, the stomach being thoroughly washed out previously.²

Winternitz Test.

Winternitz has recommended the use of iodipin instead of salol for testing the motility of the stomach. This substance is not affected by the gastric contents, but is acted upon in the intestine by the pancreatic secretion and bile in such a way that iodine is set free. This may be tested for in the saliva by adding to it a little starch paste, when a distinct blue color will be observed within 15 to 45 minutes.

VII. ABSORPTIVE POWER OF THE STOMACH

The absorptive power of the stomach is not of great importance clinically, as the greatest part of absorption occurs from the intestinal tract. However, tests for such power have a certain associated value and are, therefore, usually made. For this purpose Penzoldt and Faber advance a method depending upon the principle that under physiologic conditions potassium iodid is rapidly absorbed by the gastric mucous membrane and is immediately eliminated

¹ Thoburn and Hanzlik, Jour. Biol. Chem., 1915, XXIII, 163, outline methods for the quantitative determination of this acid in urine.

² See Boas (Deutsch. med. Wchnschr., 1912, XXXVIII, 455), Kemmerling (Arch. f. Verdauungskr., 1914, XX, 49) and Wartensleben (Ibid., 66) for a discussion of the Boas chlorophyl test.

in the saliva. A capsule containing two to three grains of potassium iodid is given to a patient shortly before a meal. The saliva is then tested as follows for the presence of potassium iodid at intervals of two to three minutes. The saliva is slightly acidified with nitric acid and treated with a few drops of starch paste when the characteristic blue color of iodid of starch will be formed by the action of the iodine liberated from the potassium iodid by the nitric acid. Under physiological conditions the first trace of iodine will appear in the saliva within ten minutes of its administration upon an empty stomach. Under pathological conditions a delayed reaction may be observed in almost all diseases of the stomach, especially in dilatation and in carcinoma. The test will naturally be delayed in case the stomach is filled with food. This test has little value, as it may appear or not in all types of gastric disease. Von Mering has found that potassium iodid is not absorbed at all from the stomach even within two or three hours, so that the iodine appearing in the saliva may be due to absorption from the intestine.¹

VIII. INDIRECT EXAMINATION OF THE STOMACH CONTENTS

As not all cases of disease of the stomach permit of examination by removal of the contents through the stomach-tube, methods have been advanced to permit of indirect determination as to the activity of the stomach contents. These methods do not permit of accurate determination of the acidity or of the ferments of the juice, but do give much information regarding the normal digestive powers and motility of the stomach.²

Günzburg's Method.

A tablet of 0.2 gram of potassium iodid is placed in a piece of the thinnest possible strongly vulcanized rubber tubing measuring about 2.5 cm. in length. The ends of the tubing are folded and the package tied with three threads of fibrin which have been hardened in alcohol. The package is now tested by placing it in warm water for several hours and examining the water for potassium iodid. The patient swallows one of these packages three-quarters of an hour after an Ewald meal, the saliva being tested for potassium iodid at intervals of 15 minutes. In the presence of free hydrochloric acid in normal amounts the threads of fibrin are dissolved and the potassium iodid is absorbed, giving a reaction in the saliva in from one to one and three-quarters hours. In cases of hypochlorhydria the reaction is delayed, a delay of six hours indicating a practical absence of free hydrochloric acid.

This test very frequently gives reliable results, but the threads of fibrin soon become brittle and break on swallowing the package so that a reaction for potassium iodid under these conditions would have no value.

Sahli's Desmoid Reaction.

Sahli has recently introduced the "*Desmoid bag*" for use in estimating the functional activity of the stomach. These bags are made of the ordinary rubber-dam used by dentists and contain a pill of 0.05 gram of methylene blue and 0.1 gram of iodoform. The bag is tied, in a manner especially

¹ See Mendel and Baumann (Jour. Biol. Chem., 1915, XXII, 165), who show that absorption is not a prominent function of the stomach.

² See Custer, Schweig. med. Wchnschr., 1921, LI, 1091 and 1115.

outlined by Sahli, with cat-gut which has been allowed to dry but has been untreated chemically. This gut, according to Sahli, is digested only by the gastric juice and not by the pancreatic juices. This pill is administered to the patient immediately following the noon meal and the urine and saliva tested at intervals of one hour, beginning three hours after administration of the pill. The digestion of the gut by the gastric juice liberates the pill and permits of the absorption of both the methylene blue and the iodoform. The methylene blue will appear in the urine coloring it green within six hours, while the iodine will be found in the saliva within two hours. Should the color of the urine not be distinctly green, this tint may be more clearly brought out by adding a few drops of acetic acid and boiling. Variations from the periods indicated above denote a hyperacidity or a hypoacidity of the gastric juice according as the time of appearance of the reactions is lessened or increased. As the gut is digested only by the gastric juice¹ a non-appearance of either reaction would indicate an anachlorhydria.



FIG. 18.—Sahli's Desmoid bag.

The writer has used these desmoid bags in a large number of cases and has found them fairly reliable, giving results which have, in many cases, been confirmed by chemical analysis. As these bags are not obtainable in the market, he has been forced to make them himself and has found that the technic of Sahli must be followed very closely, especially as regards the tying of the gut. Other methods, such as those of Dunham, Turck, and Einhorn, have been advocated, but possess no advantages over those outlined.²

IX. THE GASTRIC JUICE IN DISEASE

(1) Hyperchlorhydria.

By hyperchlorhydria is meant the secretion of an abnormally acid gastric juice whose acidity is due to an excess of free hydrochloric acid. This secretion is much more marked during digestion, being less frequent on an empty stomach. Under these conditions we usually find an increased total acidity along with the increase of free hydrochloric acid. A condition which is characterized by a high total acidity with a very high amount of organic acid would not, of course, be considered in this connection. A hyperacidity or hyperchlorhydria exists when we have more than 0.2 per cent. (60°) of free hydrochloric acid (see p. 69).

This condition may be due to pathologic changes in the mucosa or to direct nervous influences. Cases of pure hyperchlorhydria are occasionally very stubborn and may be associated with almost any variety of abnormal gastric function. While we find hypermotility of the stomach in many cases of hyperchlorhydria, we very frequently note a diminished motility due to spasm of

¹ Gregersen (Arch. f. Verdauungskr., 1913, XIX, 43) advocates the use of Schmidt's test of administering 100 grams of very slightly cooked meat and the later study of the stools for connective-tissue fiber.

² See Friedrich, Berl. klin. Wchnschr., 1913, L, 32; also, Refhuss, Am. Jour. Med. Sc. 1914, CXLVII, 848; Einhorn, Jour. A. M. A., 1919, LXXIII, 1509.

the pylorus. This condition brings about a stagnation of the stomach contents and a consequent increase in the fermentative processes. The acidity in such cases may run as high as 200° or over and the digestive powers of the gastric juice, as regards protein substances, may be much increased, the carbohydrate digestion being correspondingly diminished. These facts point to the reason for the administration of an increased protein diet in such cases, the protein combining with the hydrochloric acid and thus taking a portion of the excess from the field of action.

While this condition is not a distinct entity, yet we find many cases which come under the heading of idiopathic hyperchlorhydria and which are not associated with other pathologic conditions.¹ Some of these cases are purely functional and clear up promptly under proper treatment, while others are of nervous origin and are remedied only when the etiologic factor is eliminated. In this latter type of hyperchlorhydria the degree of acidity varies with the nervous symptoms, giving rise to the term "*heterochylia*."

(2) Hypersecretion (Gastrosuccorrhea).

By hypersecretion is meant an excessive secretion of gastric juice which is out of proportion to the physiologic stimulus.² This hypersecretion occurs even when no stimulus is present, is always pathological, and, according to Riegel, always produces pathological results. A hyper- or continuous secretion may be determined by finding a fairly large amount of gastric juice in the fasting stomach under conditions which rule out stenosis and stagnation. The stomach is washed out before the patient retires, the contents being withdrawn the following morning. If a quantity of highly acid fluid is obtained, a hypersecretion is proven. The quantity taken from the fasting stomach should never be more than 100 c.c. This secretion, to be called a hypersecretion, must contain no food remnants, no sarcinæ nor yeast cells, but should be distinctly acid.

This condition is probably a functional neurosis, being constant or intermittent and a part of a general neurosis, a secretory neurosis, or the result of organic nervous disease, such as the gastric crises of tabes dorsalis. Reichmann has reported cases of the periodic or intermittent type, during the intervals between the attack the digestion of the patient being normal. Such cases are known as Reichmann's disease.

The chronic cases are of long duration and have a gradual onset. The patient complains of much discomfort, feeling of weight or depression in the stomach, pain during digestion, vomiting, especially at night, and a gastric contents with a large amount of free hydrochloric acid. Dilatation of the stomach sooner or later comes on as the result of spasm of the pylorus induced by the hyperacidity. In these dilated stomachs we find, of course, products of fermentation and many yeast cells and sarcinæ.

(3) Achylia Gastrica.

This condition may arise either from a functional disturbance of the mucosa or a true atrophy of the mucosa. This latter state, known as atrophic

¹ See Kerley, Med. Record, 1920, XCVII, 786; Talentoni, Riv. Crit. di Clin. Méd., 1920, XXI, 385.

² See Reiss, Am. Jour. Med. Sc., 1921, CLXI, 43.

gastritis, may be the end stage of a chronic gastritis or the result of carcinoma. When this condition is not due to direct gastric disturbance, it is more frequently seen in connection with pernicious anemia, in which the general nutrition is very much below par. The local condition may not be suspected, as the not uncommon hypermotility may prevent attention being drawn to the stomach.

For a diagnosis of achylia, the test meal of Ewald gives very good results. Examination of the gastric contents shows that the food is little changed, the total acidity very low (1 to 6°), no free hydrochloric acid, gastric ferments much diminished or entirely absent, and lactic acid only in small amounts. The motility of the stomach is usually little impaired, so that the retention of food is unusual.¹

(4) Acute Gastritis.

The stomach contents of acute gastritis shows a diminished total acidity, little or no free hydrochloric acid, organic acids relatively increased, much mucus and undigested food. This condition is usually brought on by direct irritation and is generally easily remedied by total abstinence. The material for chemical examination is usually obtained in these cases from the vomitus, as the passing of the stomach-tube is very rarely tolerated.

(5) Chronic Gastritis.

All grades of this condition may exist up to complete atrophy of the mucosa.² Examination of the stomach contents shows practically no digestion of the food material, much mucus intimately mixed with the food particles, the secretion usually diminished, free hydrochloric acid diminished or absent, ferments much reduced, protein digestion small, starch digestion little affected, microscopic examination showing the presence of many epithelial cells and leucocytes. There are some cases of chronic gastritis in which a hyperacidity of the juice is evident, but these are rare, the usual finding being one of diminished acidity. The motility of the stomach in these cases is sometimes normal, sometimes increased, or may be diminished. One of the most characteristic findings in this condition is the presence of a large amount of mucus containing either leucocytes or their nuclei and epithelial cells from the walls of the stomach. If there is little acid present in the gastric contents the mucus may swell up and appear greater in volume. As a general rule, it may be said that the mucus and the hydrochloric acid vary inversely as their amounts.

(6) Nervous Dyspepsia.

This condition of nervous dyspepsia is part of a general neurosis and may show no characteristic findings in the stomach contents. The degree of acidity may range from a normal to either a hyper- or a hypoacidity, while the

¹ Disqué (Arch. f. Verdauungskr., 1914, XX, 366); Orloff (Russk Vrach., 1914, XIII, 1006) believes there eventually occurs a vicarious increased secretion of trypsin in this condition. See, also, Rehfuß, Am. Jour. Med. Sc., 1915, CL, 72; Andresen, Med. Record, Beck and McLean, Southern Med. Jour., 1919, XII, 594.; 1916, LXVII, 1629; Rydgaard, Hospitalstid., 1920, LXIII, 2 and 17; Faber, Arch. f. Verd.-Kr., 1920, XXVII, 24; Willemse, Nederl. Tijdschr. v. Geneesk., 1921, II, 3069; Fischbein, Boston, Med. & Surg. Jour., 1922, CLXXVI, 413; Golob, Med. Record, 1922, CI, 540.

² See Rehfuß, Penn. Med. Jour., 1921, XXIV, 233.

amount of ferments present will not usually vary. The findings in nervous dyspepsia are not at all constant, varying at different examinations. We have, therefore, more or less distinct methods of differentiation between this condition and chronic gastritis. In the first place, the acidity of chronic gastritis remains constant for several examinations while that of nervous dyspepsia is variable. The ferments are diminished in cases of gastritis, while they are normal in nervous dyspepsia. Much mucus is found in gastritis while little or none is present in dyspepsia. The cases of nervous dyspepsia are partially associated with distinct errors in eating. The influence of nervous conditions over gastric function has been very well expressed by Emerson when he says, "a neurasthenic will often worry his subliminal gastric sensations into the sphere of consciousness."

(7) Ulcer of the Stomach.

The diagnosis of ulcer of the stomach depends, to a large extent, upon the clinical symptoms of the disease rather than upon the examination of the stomach contents.¹ While the symptoms of this disease, increasing dyspepsia, pain, vomiting, blood in the vomitus, and hyperacidity of the vomitus, are well-known, the stomach-tube should rarely ever be used in obtaining the contents of the stomach, owing to the danger of perforation in such cases.²

The vomitus of such cases is usually ejected from one to three hours after a meal and contains well-digested food. Blood may or may not be present and may be either fresh red blood or dark in color from the formation of hematin. The total acidity of the gastric contents is usually increased, hydrochloric acid constituting a large part of this total acidity. A single examination of the gastric contents will rarely determine anything about an ulcer, so that repeated examinations of the vomitus must be made to obtain a general idea of the acidity. In these cases blood is usually present in the feces and may be detected as outlined later.³ When an ulcer is complicated by a beginning carcinoma, we may find all types of variation in the acidity of the stomach contents.

(8) Carcinoma of the Stomach.

In no other condition of the stomach is an absolutely certain early diagnosis to be so much desired as in carcinoma of the stomach. The chemical

¹ See Verbrycke, *Am. Jour. Med. Sc.*, 1913, CXLVI, 742.

² Rosenow (*Jour. Am. Med. Assn.*, 1913, LXI, 1947) has shown the close relationship of streptococcic invasion to gastric ulcer. See, also, Rosenow and Sanford, *Jour. Infect. Dis.*, 1915, XVII, 219; Rosenow, *Ibid.*, 1916, XIX, 333; Bolton, *Jour. Path. and Bacteriol.*, 1915, XX, 133; Wilensky and Geist, *Jour. A. M. A.*, 1916, LXVI, 1382; Steinharter, *Boston Med. & Surg. Jour.*, 1916, CLXXIV, 678; CLXXV, 59; 1917, CLXXVI, 401; Hardt, *Am. Jour. Physiol.*, 1916, XL, 314; Celler and Thalheimer, *Jour. Exper. Med.*, 1916, XXI, 791; Dragstedt, *Jour. A. M. A.*, 1917, LXVIII, 330; Smithies, *Ill. Med. Jour.*, 1917, XXXI, 149.

³ See Lichty, *Am. Jour. Med. Sc.*, 1914, CXLVIII, 680; Smith, *Jour. Med. Research*, 1914, XXX, 147; Smithies, *Arch. Diagnosis*, 1915, VIII, 147; Interstate Med. Jour., 1915, XXII, 207; Boas, *Arch. f. Verdauungskr.*, 1915, XXI, 94; Sippy, *Jour. Am. Med. Assn.*, 1915, LXIV, 1625; Mayo, *Ibid.*, 1915, LXV, 1069; Cheney, *Ibid.*, 1915, LXV, 1227; Stone, *Jour. A. M. A.*, 1916, LXVI, 324; Dahl, *Hygiea*, 1916, LXXXVIII, 1408; Moschowitz, *Am. Jour. Med. Sc.*, 1916, CLII, 714; Ivy, *Arch. Int. Med.*, 1920, XXV, 6; Fricker, *Schweiz. med. Wchnschr.*, 1920, L, 63; Dahl, *Hygiea*, 1920, LXXXII, 159; Campos, *Siglo. Méd.*, 1920, LXVII, 323 and 364; Fricker, *Schweiz. med. Wchnschr.*, 1921, LI, 174; Haudek, *Wiener klin. Wchnschr.*, 1921, XXXIV, 159; Gundermann and Düttmann, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1921, XXXIII, 480; Lutz, *Arch. f. klin. Chir.*, 1921, CXV, 780; Meyenburg, *Münch. med. Wchnschr.*, 1921, LXVIII, 633; Birt, *Deutsch. Ztschr. f. Chir.*, 1921, CLXV, 1.

features of the gastric juice in this condition may be very suggestive or may be negative. The clinical history of the case along with the age of the patient are probably of more importance in making a diagnosis of carcinoma than are variations in the chemical composition of the juice. The local symptoms of carcinoma of the stomach are sometimes as variable as are the changes in the gastric juice, so that every possible point in diagnosis should be taken advantage of, if for no other purpose than to exclude this condition.¹

Perhaps the most important sign of carcinoma is the absence of free hydrochloric acid. Although this condition is present in about 85 per cent. of cases, it cannot always be traceable to carcinoma, as it may occur in atrophic gastritis and advanced chronic gastritis. This lack of free hydrochloric acid is due to the union of this acid with some body which in itself does not show an alkaline reaction. Von de Velden suggests that the secretion from the cancer is the active agent in neutralizing the hydrochloric acid. Moreover, the products of protein digestion might have some power in neutralizing the acid, Emerson having shown that hexone bases are present as a result of the action upon the protein of a ferment² derived from the tumor itself. Certainly, in cases of carcinoma the total nitrogen of the stomach contents is much increased, so that such bodies are probably a very great factor in the diminution of the hydrochloric acid. This reduction in hydrochloric acid is also influenced by changes in the mucosa to such an extent that the active secretion is diminished. The failure of free hydrochloric acid is usually a very early symptom, but it must be remembered that hydrochloric acid may be present in normal amounts or even in increased amounts when the carcinoma is small and occupies the pyloric region or when this growth develops on the base of an old ulcer.³ The acidity may vary markedly from day to day, sometimes showing no free hydrochloric acid and sometimes a considerable amount. This variation is of considerable practical importance. In cases of carcinoma of the esophagus the disappearance of hydrochloric acid from the gastric contents seems necessarily to be the result of the secretion of the tumor neutralizing the gastric juice. Along with this diminution in free hydrochloric acid the total acidity is also diminished.⁴

The presence of an increased amount of lactic acid is a very valuable sign in cancer.⁵ About 90 per cent. of patients show the presence of lactic acid when there is no free hydrochloric acid, but when there is a large amount of combined HCl pointing to a free secretion of this acid. Lactic acid may not be present in cases of carcinoma, especially those in which the growth is upon the base of an old ulcer, or it may be present in conditions other than carci-

¹ See Schütz, *Wien. klin. Wchnschr.*, 1913, XXVI, 1053, and *Arch. f. Verdauungs-Kr.*, 1915, XXI, 421; Patella, *Rif. Med.*, 1916, XXXII, 1, 29, 57 and 85; Kahn, *Jour. Lab. and Clin. Med.*, 1916, II, 103.

² Kriloff (*Med. Obozr.*, 1913, LXXIX, 1) believes trypsin is always present in cancer, especially of the pylorus. See, also, Halpern, *Mitt. a. d. Grenzgeb. der Med. u. Chir.*, 1914, XXVIII, 709. Pron, *Compt. rend. soc. biol.*, 1916, LXXXIX, 68.

³ See Levy, *Beitr. z. klin. Chir.*, 1914, XCIII, 696; Ochsner, *Jour. Am. Med. Assn.*, 1915, LXV, 1973; Zoppelitz, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1916, XXIX, 1; Hartman, *Am. Jour. Med. Sc.*, 1922, CLXIII, 186.

⁴ See Friedenwald, *Am. Jour. Med. Sc.*, 1914, CXLVIII, 660; Smithies, *Jour. Am. Med. Assn.*, 1914, LXIII, 1839; *Ibid.*, 1915, LXIV, 643.

⁵ See Schryver and Singer, *Quart. Jour. Med.*, 1913, VI, 71 and 309.

noma, such as chronic gastritis, associated with atrophy of the mucosa and dilatation of the stomach, especially when a benign stenosis of the pylorus exists. It must be stated, however, that we usually find an increased secretion of hydrochloric acid in cases of simple benign pyloric stenosis, so that lactic acid cannot be formed in the presence of this increased hydrochloric acid. According to Riegel, the chief cause of the lactic acid formation is the combination of motor insufficiency with a hypoacidity associated with a diminished secretion, both of acids and ferments. This diminution in the amount of ferments is in no way specific for cancer, as it is in reality due to the chronic gastritis which is set up by the tumor. As lactic acid cannot be formed in the presence of an appreciable amount of free HCL, we do not always find after a test meal a large excess of this acid, especially in those cases in which considerable combined HCL is present. It seems advisable, therefore, in testing for the presence of lactic acid, to examine the contents of the fasting stomach in the morning after it has been well washed out the preceding evening. Whether we are to assume that lactic acid is formed by the action of the organisms in the stomach upon food material, whether it be a normal product of digestion, or whether it is a product of the activity of a ferment derived from the tumor, must be left for future investigation.

The vomited material or the material obtained by washing out the stomach shows very little digestion of the protein elements, while the carbohydrates are well hydrolyzed. The amount of material in the stomach will be large or small depending upon the degree of stenosis, so that it is nothing unusual to obtain several pints of material containing undigested protein residue. Microscopic examination of this material may show cellular masses washed off from the tumor or may show these fragments embedded in masses of blood.¹ Blood is a usual finding in cases of carcinoma and may be detected by the methods discussed under *Feces*. *Sarcinæ* and yeasts are rare, the former occurring more frequently in cases of marked dilatation. The *Boas-Oppler* bacilli are more or less constant findings in carcinoma, occurring in about 90 per cent. of the cases and only very rarely in any other condition.

Salomon's Test.

This test is based upon the fact that albumin is secreted from the carcinoma itself and passes into the gastric contents. The patient is placed upon an absolutely protein-free diet for 24 hours and the stomach carefully washed out at the end of this time with 400 c.c. of physiological salt solution. A few hours thereafter the contents of the stomach are removed and the remaining material washed out with 400 c.c. of physiological salt solution. The total nitrogen and the albumin are then estimated, the former by the Kjeldahl method and the latter by the Esbach method (see *Urine*). The nitrogen ranges from 10 to 70 mg. per 100 c.c. in cases of carcinoma, while in other conditions it varies from 0 to 16 mg. per 100 c.c. The Esbach reaction gives a distinctly appreciable precipitate for albumin, anything over 0.5 parts per 1000 being considered indicative of carcinoma. While this test is not infallible,

¹ See Simon and Caussade, *Presse méd.*, 1914, XXII, 265; Loeper and Binet, *Arch. des Mal. de l'App. digestif*, 1914, VIII, 181; Caussade, *Rev. de Med.*, 1914, XXXIV, 428.

yet the writer has found it present in so many cases of carcinoma that he is inclined to make a very strong presumptive diagnosis on the basis of this test.¹

Neubauer and Fischer's Test.

These investigators,² working in Müller's clinic, have taken advantage of the well-known fact that normal peptic digestion does not proceed to the formation of amino-acids but stops at the peptone or peptid stage. Further, it has been shown by Emerson, Fischer and others that digestion of protein goes somewhat further in carcinomatous stomachs than in normal ones, owing to the fact that carcinomatous tissue, as well as various body tissues, contains proteolytic ferments capable of producing more complete hydrolysis than does pepsin (see Lewin³). Neubauer and Fischer, following the investigations of Erdmann and Winternitz, Glaessner, and Volhard, use this hydrolysis of peptids by such proteolytic ferments for diagnostic purposes. Their method is as follows: The contents of the stomach are withdrawn one-half to three-fourth hour after an Ewald meal; 10 c.c. of the filtered material are mixed with a little glycyl-tryptophan⁴ and a little toluol is added to prevent bacterial action. The mixture is placed in the incubator at 37°C. for 24 hours. At the end of this period, withdraw 2 or 3 c.c. of the mixture from beneath the toluol layer, place this in a test-tube, add a few drops of 3 per cent. acetic acid and then allow the fumes of bromin to pass into the tube from an open bottle of bromin. This part of the technic must be carried out very carefully as an excess of bromin will obscure and even destroy the reaction. After shaking the tube a positive reaction is shown by the appearance of a rose-red or intense red color. If no red color appears, carefully add more bromin vapor and shake. This addition may be continued until an excess of bromin is evidenced by a light yellow color of the mixture.

These workers have shown that errors may arise in this test due to the presence of (1) tryptophan in the gastric contents, (2) peptid-splitting bacteria, (3) trypsin or pancreatic juice, and (4) blood. To do away with these sources of error they advise the rejection of gastric contents showing any of these substances. Filtering the contents removes the peptid-splitting bacteria to some extent while the addition of toluol prevents their development. Tryptophan, as such, may be tested for as usual, while the presence of pancreatic juice may be assumed if bile be present. Blood is shown by tests outlined under Feces. Warfield⁵ has recently shown another fallacy of this test, which may render the test of little clinical value. He has found that the saliva of many patients is capable of decomposing glycyl-tryptophan into its constituent elements. He concludes, therefore, that swallowed alkaline saliva, when mixed with neutral or faintly acid (not over 0.05 per cent. HCl) gastric contents, may introduce sufficient error to account for the discordant

¹ See Hohlbaum, Arch. f. klin. Chir., 1914, CIV, 1069.

² Deutsch. Arch. f. klin. Med., 1909, XCVII, 499.

³ Ergebn. d. inn. Med., 1908, II, 212.

⁴ Glycyl-tryptophan, as prepared for the test, is a clear solution. It is furnished by Kalle & Co., Biebrich a. Rhein, under the name of "Fermentdiagnostikum." It comes in small bottles which are used directly for the test by simply adding the 10 c.c. of filtered gastric contents and incubating for 24 hours.

⁵ Bull. Johns Hopkins Hosp., 1911, XXII, 150.

results obtained. Koelker¹ has found a di- and tripepid-splitting ferment in the saliva. Lyle and Kober² believe that a negative result with this test is of far greater value than is a positive one.

Weinstein³ believes that the presence of tryptophan in the gastric contents, as withdrawn, is more positive as a test for carcinoma than is the glycyl-tryptophan test, providing intestinal fluid has not regurgitated into the stomach. He has confirmed the findings of Warfield and asserts that this latter fallacy can not be applied to his "tryptophan test" as the salivary proteolytic action is not such as to produce tryptophan from protein in non-cancerous gastric contents. He, therefore, suggests the following modification. A portion of the gastric contents is withdrawn 4 to 5 hours after a full dinner and the filtered contents tested directly for tryptophan after acidifying the filtrate with acetic acid. If no reaction appears, incubate for 24 hours and repeat the test. Owing to the large number of possible fallacies and the discordant results obtained with this test, it seems probable that it, like so many others, must be regarded as of little diagnostic value.⁴

Freidman and Hamburger (Arch. Int. Med., 1913, XII, 346) believe that the proteolytic cleavage of stomach contents is due, in most instances, to regurgitated trypsin, although leucocytes and bacteria play some rôle. They advocate a combined method of proteolysis and peptolysis, which they believe preferable to other tests advanced. A high peptolysis with low proteolysis speaks for carcinoma; while a high peptolysis and high proteolysis speaks against carcinoma. This test promises to be a reliable one but confirmatory work must establish its status.

Wolff and Junghans' Test.

These workers⁵ have originated a test for the differentiation of benign and malignant achylas, which test seems to have considerable diagnostic value if properly controlled and interpreted. The test is based on the following observations. Normal aspirated test meals show a relatively large amount of soluble albumin, which arises from the digestion of the protein of the test meal by the gastric ferments in the presence of free hydrochloric acid. This albumin increases in amount in proportion to the time which it remains in contact with the normal gastric juice. In conditions associated with achylia or with marked diminution of hydrochloric acid in the stomach, it would be reasonable to suppose that little or no protein digestion would occur. This was found to be the case in benign achylas but not true in the malignant types. In these

¹ Ztschr. f. physiol. Chem., 1911, LXXVI, 27.

² New York Med. Jour., 1910, XCI, 1151.

³ Jour. Am. Med. Assn., 1910, LV 10,85; Ibid., 1911, LVII, 1420.

⁴ In this connection see Taylor and Hall, Jour. Path. and Bacteriol., 1912, XVII, 121; Sanford and Rosenbloom, Arch. Int., Med., 1912, IX, 445; Smithies, Ibid., 1912, X, 357 and 521; Jacque and Woodyatt, Ibid., 560; Hamburger, Jour. Am. Med. Assn., 1912, LIX, 847; and Smithies, Ibid., 1913, LXI, 1793.

⁵ Wolff and Junghans, Berl. klin. Wehnschr., 1911, XLVIII, 978; Wolff, Magen und Darmkrankheiten, Berlin, 1912, 217. See, also, Thiele, Berl. klin. Wehnschr., 1912, XLIX, 544; Einstein, Med. Klin., 1912, VIII, 484; Roubitschek and Weiser, Arch. f. Verdauungskr., 1913, XIX (Erganz. Bd.), 146; Rolph, Med. Record, 1913, LXXXIII, 848; Smithies, Am. Jour. Med. Sc., 1914, CXLVII, 713; Trallero, Deutsch. med. Wehnschr., 1914, XL, 1428; Kahn and Jacobowitz, Biochem. Bull., 1915, IV, 214; Lenskaia, Russk. Vrach., 1915, XIV, 267; Katznelson, Ibid., 292; Clarke and Rehfuss, Jour. Am. Med. Assn., 1915, LXIV, 1737. Friedenwald and Kieffer, Am. Jour. Med. Sc., 1916, CLII, 321.

latter cases the soluble albumin (probably a proteose) was found to be much increased. This albumin may arise (1) from protein retention from a previous meal, due to stasis, lack of motor tone, or true obstruction, as shown by Clarke and Rehfuß; (2) from mixing of the test meal with so-called "cancer juice" exuded from the malignant growth; (3) from the presence of blood or pus arising from ulcerative lesions; (4) from the presence of swallowed saliva or, especially, sputum; and (5) from the action of a specific peptid-splitting ferment arising from the malignant growth and capable of splitting the protein into lower groupings of aminoacids. This latter source is the most important, although the others must be excluded before proper interpretation is possible. In other words, one must be sure (1) that the stomach is free from remnants of previous protein meals, as in some cases this gastric residuum may be sufficient to give erroneous results;¹ (2) that blood and pus are absent, at least in more than traces; and (3) that sputum especially is not swallowed during the period of retention of the test meal, as some types of sputum are relatively rich in soluble albumins.

While, theoretically, typical cases of advanced gastric carcinoma, at least, should show little or no free hydrochloric acid, it should be remembered that such is actually not the case, so that this test applied to cases in which free hydrochloric acid is present to an appreciable extent may, of course, give high albumin values due to ordinary gastric digestion. However, the studies of Clarke and Rehfuß, by means of their fractional method of gastric examination, show that the curve of protein digestion under normal conditions follows the acid curve rather sharply, while in malignant states the protein curve diverges from the acid curve and, as digestion progresses, this disproportion increases until there is a marked separation of the curves. From these facts, these workers argue that the fractional study of both the curves of gastric acidity and protein digestion should be simultaneously studied before the proper interpretation can be reached.

Technic.

In order to avoid the possibility of retention of protein residues in the stomach, the patient is allowed a light meal with very little protein on the evening before the examination is to be made. A light cathartic is administered the same evening. The next morning administer an Ewald test breakfast. Withdraw this meal within 45 to 60 minutes. Filter the contents through filter-paper until clear.

While filtration is proceeding, arrange a series of six clean test-tubes in a rack, numbering the tubes serially. By means of a 1 c.c. pipet, which is graduated in divisions of 1/100 c.c., place in the respective tubes 1 c.c., 0.5 c.c., 0.25 c.c., 0.1 c.c., 0.05 c.c., and 0.025 c.c. Now make up the volume in each tube to 10 c.c. by the addition of distilled water from a finely graduated pipet. The dilutions in these tubes will be, then, 1 to 10, 1 to 20, 1 to 40, 1 to 100, 1 to 200, and 1 to 400. Intermediate and extra dilutions may be made if it is desired to work the reaction down to a much finer point. Mix the contents of the tubes thoroughly by shaking and inverting the tubes.

¹ See Rehfuß, Bergeim and Hawk, Jour. Am. Med. Assn., 1914, LXIII, 11.

Overlay the contents of each tube with 1 c.c. of the reagent to precipitate the albumin and note the appearance of a white ring at the point of contact of the fluid and reagent in the various tubes. A black background will bring out these rings more distinctly.

Wolff's reagent is as follows:

Phosphotungstic acid	0.3 gram.
Concentrated Hydrochloric Acid.....	1.0 c.c.
Alcohol (96 per cent.).....	20.0 c.c.
Distilled Water.....	ad 200.0 c.c.

Interpretation.

Normally a distinct ring of albumin will be seen at dilutions of 1 to 10, 1 to 20 and, occasionally, at 1 to 40. This reaction, which is called negative at these dilutions, may even reach, in very rare cases, as high as 1 to 200 due to various factors spoken of above, especially to the presence of blood and retention of protein residues. The importance of excluding extrinsic sources of albumin is thus evident. Generally speaking, however, the diagnosis of cancer becomes more probable the higher the dilution at which the albumin precipitation occurs, although cancer cannot be excluded in some cases when the precipitation does not appear in the last tubes. A precipitation unit of 100 or over speaks rather markedly for a diagnosis of cancer, presumption becoming almost a relative certainty when the unit is 200 to 400. This implies, of course, that all the points mentioned above have been taken into consideration. Smithies regards a reaction at a dilution of 100 as doubtful. He states that this test "was a more constant finding in gastric extracts than were absent free hydrochloric acid, the presence of lactic acid and the glycytryptophan test. It was rather more constant than tests for occult blood and the demonstration of motor inefficiency. It was not so consistent in its manifestation as the demonstration of organisms of the Boas-Oppler group or the increase in the formol index."

It will be seen, therefore, that this test, of positive precipitation of albumin in dilutions of the gastric extract from 1 to 200 up, is of considerable value in the differentiation of the benign and malignant achylas or achlorhydrias, especially if the former are not associated with motor insufficiency. In cases of ulcer, especially if pyloric stenosis obtains, positive Wolff-Junghans' tests are not infrequent, indicating that symptomatology, gastric acidity and other findings must be taken into consideration. Like many other tests for cancer, this must not be relied upon to settle the diagnosis, to the exclusion of other laboratory and clinical data. With proper technic and care in excluding extrinsic factors, this test has certainly more than a presumptive value, giving definitely positive findings in fully 80 per cent. of disputed cases.

CHAPTER IV

THE FECES

I. GENERAL CONSIDERATIONS

The feces are composed of substances of different origin, which may be divided as follows: (1) Food remnants, either undigestible constituents or digestible but unabsorbed elements; (2) secretions of the alimentary tract; (3) decomposition products and bacteria; (4) formed and unformed elements derived from the intestinal wall; and (5) foreign bodies, such as hair, wood fiber, parasites, parasitic ova, and enteroliths. It is not possible to draw a sharp line between a truly physiologic and pathologic composition of the feces. In each individual case this division will depend upon a number of factors, among which may be mentioned composition of the food, method of taking the food, individual functional capacity of the intestines, frequency of the bowel movements, and general systemic conditions. The condition of the food remnants will give much information regarding the functional capacity of the bowel.¹ Under normal conditions, about one-third of the dry substance of the feces arises from the bacteria, this amount increasing under certain pathologic conditions which will be later discussed. As products of bacterial activity we observe the formation of hydrobilirubin from bilirubin as well as the reduction of certain medicaments and the rare formation of certain diamins, which will be treated later. This bacterial activity will vary much under pathologic conditions and may have much to do with the symptomatology of the case investigated.² The ordinary decomposition products are derived from the carbohydrates and proteins of the food; from the former are produced, by fermentation, volatile fatty acids, lactic acid, succinic acid, alcohol, carbon-dioxid, hydrogen, and methane, while from the latter are formed by putrefaction indol, skatol, phenol, ammonia, and hydrogen sulphid. The fats are decomposed only to a very slight extent. The normal products of digestion in the intestinal tract as well as the factors bringing about these changes will be discussed later.³

Normal Feces.

For proper comparisons between the feces of various individuals the food must be the same. Starvation feces, meat feces, and milk feces are typical types, but are not normal in the strict sense. For comparison, however, an arbitrary norm must be established in order to judge of slight variations which have to do with special differences in utilization of food and which are not observable by the eye except under certain conditions (as, for instance, fatty

¹ See Drueck, *Ill. Med. Jour.*, 1920, XXXVIII, 130; Burnett, *Boston Med. & Surg. Jour.*, 1921, CLXXXIV, 415; Coops, *Lancet*, 1921, II, 9.

² See von Noorden, *Jour. Am. Med. Assn.*, 1913, LX, 101.

³ See Sanford, *Wisconsin Med. Jour.*, 1914, XII, 281; also, McClanahan and Moore, *Am. Jour. Med. Sc.*, 1915, CXLIX, 815; Bergeim, *Jour. Biol. Chem.*, 1917, XXXII, 17.

stools). Through the work of Praussnitz and of Schmidt and Strasburger a new field for study of normal and abnormal feces has been opened up.

A normal feces should be one which consists almost entirely of remnants of the digestive juices and intestinal secretion arising from a purely digestible, properly prepared, and assimilable food. It contains approximately 8.6 per cent. of nitrogen, 16 per cent. of ether extract, and 15 per cent. of ash calculated on the dry basis. Any appreciable variation from this composition would indicate a diminution of the functional activity of the bowel. In the investigation of pathological cases this composition is scarcely to be assumed, as the diet is more or less restricted, the appetite is capricious, the intestinal activity is variable, and the feces are, therefore, different from those of the healthy. It must not be understood from this that a feces showing the



FIG. 19.—Normal Feces. (Landois.)

a, Muscle fibers; *b*, tendon; *c*, epithelial cells; *d*, leucocytes, *e-i*, various forms of plant-cells, among which are large numbers of bacteria; between *h* and *b* are yeast-cells; *k*, ammonium-magnesium phosphate.

above composition is necessarily normal in other respects. One may not rely entirely on the chemical composition, but must largely consider the macroscopic and microscopic findings. In order that we may have a sound basis upon which to judge of intestinal activity it seems wise to have some sort of test diet which may be given to suspected cases.

Diet of Schmidt and Strasburger.

This diet¹ is so selected that it can be used by the healthy as well as by those with intestinal trouble; its amount is sufficient to satisfy the maximal caloric requirement of the individual while at rest; it contains the three chief groups of food material in definite relation to each other; is as free as possible from remnant-leaving food, and can be easily obtained and prepared. The starchy food present is of the amount and kind which have been shown most favorable for the prevention of excessive fermentation in the bowel. The daily diet is as follows: One and five-tenths liters of milk, 100 grams of zwieback, two eggs, 50 grams of butter, 125 grams of beef (raw weight), 190 grams cooked potato, and a gruel of 80 grams of oatmeal. This is distributed

¹ Die Fäces des Menschen, Berlin, 1915.

through the day as may best suit the patient. This diet contains 102 grams of protein, 111 grams of fat, and 191 grams of carbohydrate, yielding 2,234 raw calories. In cases which show a diarrhea, due to the milk, one may substitute instead of $\frac{1}{2}$ liter of milk the same amount of cocoa made from 20 grams of cocoa powder, 10 grams of sugar, 100 grams of milk, and 400 grams of water. Small variations in the amount of milk, sugar, butter, and even of eggs may be permitted, but the outline, as regards meat, zwieback, potato, and gruel should be rigidly adhered to.

This diet should be administered for three days or longer if necessary to obtain a stool which comes from it. In order to judge of the first appearance of the stool from this diet, the patient should be given a capsule containing 0.3 gram of powdered carmin, both preceding and following the diet.¹ Instead of carmin, one may use cork, charcoal, or silicic acid.

Folin's Diet.

This diet² is especially serviceable in case one wishes to follow the metabolism in any special case. Its easy application and its fairly constant values for nitrogen, phosphorus, chlorin, and sulphur make it invaluable.

The standard diet, which is given to the patient daily for several days, is as follows:

Whole milk,	500 c.c.
Cream (18 to 22 per cent. fat),	300 c.c.
Eggs (whole),	450 gm.
Horlick's malted milk,	200 gm.
Sugar,	20 gm.
Sodium chlorid,	6 gm.
Water,	2,100 c.c.

This diet contains approximately 119 gm. of protein, 148 gm. of fat, and 225 gm. of carbohydrate, yielding 2787 raw calories. The intake of one day is nitrogen 18.9 gm., 5.9 gm. of P_2O_5 , 3.8 gm. of SO_3 , and 6.2 gm. of Cl. It is not to be compared with that of Schmidt and Strasburger for estimating the intestinal activity, but is more reliable if metabolism relations are to be studied.

The normal motility of the feces is from 6 to 20 hours, while on a milk diet it may vary from 36 to 48 hours. This factor may be of importance in judging of intestinal obstruction. The isolation of a stool under a special diet is of great importance, as the success of an investigation will depend upon this.

Obtaining Intestinal Contents.

In the past the duodenal contents have been obtained by extremely crude and totally unreliable methods, such as massaging the abdomen and collecting, by means of the stomach tube, the material which had passed from the

¹See Basch, Jour. Am. Med. Assoc., 1913, LXI, 1295; Strauss, Arch. f. Verdauungskr., 1914, XX, 299; Hymanson, Am. Jour. Dis. Child., 1916, XI, 112.

²Jour. Physiol., 1905, XIII, 45.

duodenum into the stomach or, more recently, by the administration of an oil test-breakfast of 150 c.c. of olive oil when the stomach was empty and the removal of the gastric contents $\frac{3}{4}$ hour thereafter.

Fortunately we now have reliable methods for this work, which permit of an exact study of the conditions obtaining in the duodenum during fasting and during digestion. By the use of the Einhorn or the Rehfuß tube the duodenal contents may be withdrawn and submitted to the various tests desired. The Rehfuß tube has the advantage that it may first be used to withdraw the gastric contents and then allowed to pass into the duodenum. This latter process is facilitated by having the patient turn on his right side with the left knee flexed over the right. The time taken for the tube to pass into the duodenum varies, but averages about $\frac{1}{2}$ hour after the time when the fractional gastric analysis is finished. After the tube has reached the duodenum, the contents may be withdrawn fractionally, just as in the case of the gastric contents.

The contents of the normal fasting duodenum are usually free from bile and, hence, show a pearly gray color, although at times this color may be slightly yellow. The fluid is usually of a syrupy consistency and is nearly transparent showing only a slight sediment. The reaction of the duodenal fluid is usually slightly alkaline ($P_H = 7.7$) although it may be neutral or, even, faintly acid. This question of the reaction of the intestinal contents is one, which has been subject to the general impression that the reaction is always alkaline during digestion. While this may be true in some cases, it is not by any means true in all. The degree of alkalinity is usually highest in the fasting state and falls immediately after the test meal is given and then rises. Normally there appears to be no relation of the variation in alkalinity to the curve of gastric digestion. Myers and McClendon report that the reaction of the duodenal contents between 3 and 4 hours after meals usually fluctuates around the neutral point but that the extreme range on the acid side is greater than on the alkaline side, the reaction ranging from P_H 3.2 to 7.82. On the basis of their results showing, that the P_H of the duodenal contents ranged from 4.1 to 6.5, McClendon, Bissell, Lowe and Meyers stated that "Our object in this paper is—to discuss the causes of the prevalent erroneous impression that the intestinal content is alkaline." The figures obtained by McClure, Wetmore and Reynolds agree fairly well with those just given, the range being between P_H 5 and 7.5 with an occasional exception between 4 and 5. Recently Okada and Arai have shown, in a series of 12 cases, that the duodenal contents during digestion were acid in 7 cases, alkaline in 4, and neutral in 1.

While the above figures were obtained from the examinations of normal individuals, Einhorn reports a series of 40 cases of gastric ulcer, in which 16 showed at times an acid state in the duodenum, either in the fasting condition or at some period of the fractional examination, 2 showed a steady acidity in the entire period of fractional examination, and 24 showed constant alkalinity. It will be seen, therefore, that the reaction of the intestinal contents varies in both normal and pathologic conditions, so that it is a difficult

matter to draw any hard and fast conclusions as to the value of such determinations.¹

Obtaining Bile from Duodenum (Meltzer-Lyon Method).

Lyon, following the observation of Meltzer that a solution of magnesium sulphate, applied to the duodenal mucosa, caused a relaxation of the sphincter of the duodenal outlet of the common duct with possible contraction of the gall-bladder musculature, has devised a method for the obtaining of bile from different portions of the biliary tract for examination.

After the mouth of the patient has been thoroughly rinsed with an anti-septic solution, a sterile Rehfuß tube is passed into the stomach and the fasting interdigestive residuum removed, after which the stomach is washed out. The patient then turns on his right side, with his left knee flexed over his right, and the tube is allowed to pass into the duodenum, a process which requires from $\frac{1}{2}$ to 1 hour. After being certain that the tube is in the duodenum (which may be told by the duodenal tug detected by gently pulling the tube and by the character of the fluid aspirated), a connection is made with a sterile aspirating vacuum bottle and the duodenal contents removed. When this fluid is withdrawn, the first bottle is detached and 50 c.c. of warm sterile 25 per cent. solution of magnesium sulphate solution are injected by means of a sterile syringe. The tube is now attached to a second bottle and gentle aspiration started. In from 2 to 10 minutes bile is obtained, which is mixed with the $MgSO_4$ solution. When the color deepens to a distinct yellow, the bottle is detached and a third sterile bottle attached and drainage kept up. This first bile is probably derived only from the common duct, is from 10 to 20 c.c. in amount, may be transparent, and is light in color. After a variable period of 10 to 30 minutes, the bile becomes darker and more viscid. This is the gall-bladder bile and varies normally from 25 to 75 c.c. in amount, is neutral or faintly alkaline in reaction, has a specific gravity of 1015 to 1030, and is deep yellow in color. Following this stage the bile again assumes a lighter color, is thinner, is alkaline in reaction, has a specific gravity of 1008 to 1010, is transparent and flows more slowly and intermittently. When this change from a dark to a lighter bile is noted, a further sterile bottle is attached and this last type collected separately. This final bile is freshly secreted from the liver.

After collection of these different specimens, they may be studied as to color, viscosity, reaction, specific gravity, turbidity and sediment. A microscopic examination is made for cellular elements, such as epithelial cells, pus cells and blood cells. Finally bacteriological studies are made to isolate any organisms present. This method promises much in the study of various infections of the biliary tract and appears to be especially instruc-

¹ Einhorn, *Am. Jour. Med. Sc.*, 1918, CLVI, 817; Buckstein, *Jour. Am. Med. Assoc.*, 1919, LXXIII, 670; *Ibid.*, 1920, LXXIV, 664; Humbert, *Ibid.*, 1920, LXXV, 1423; Myers and McClendon, *Jour. Biol. Chem.*, 1920, XLI, 187; McClendon, Bissell, Lowe and Meyer, *Jour. Am. Med. Assoc.*, 1920, LXXV, 1638; Mauban, *Rev. de Med.*, 1921, XXXVIII, 146; McClure, Wetmore and Reynolds, *Jour. Am. Med. Assoc.*, 1921, LXXVII, 1468; Friedenwald and Sandler, *Ibid.*, 1469; Einhorn, *Ibid.*, 1471; Okada and Arai, *Jour. Biol. Chem.*, 1922, LI, 135.

tive in cholecystitis.¹ Bacteriological examination should prove especially valuable in detection of typhoid carriers.

Trypsin is best detected by the method of Gross.² Either the fluid obtained by the Rehfuß tube or a portion of the feces, rubbed up with three times its amount of a 1 to 1,000 Na_2CO_3 solution and filtered, may be used for the test. 100 c.c. of a 0.5 per thousand solution of casein in 1 to 1,000 Na_2CO_3 solution are treated with 10 c.c. of fluid mentioned above and the mixture placed in the incubator for eight to twelve hours. At the end of this period the addition of a few drops of a dilute (1 per cent.) acetic acid should produce no precipitate in case digestion is complete, that is if trypsin is present.

Functions of the Intestinal Juices.

The pancreatic juice as excreted into the intestine is an alkaline fluid³ containing three ferments, trypsin which hydrolyzes protein, amylopsin which acts upon the carbohydrates, and lipase (steapsin) which aids in the digestion of fat. The trypsin appears to be excreted in the form of a zymogen which is activated by a second ferment, enterokinase, derived from the intestinal mucosa, while the lipase and amylopsin are active when secreted. It has been shown by Pawlow that the passage of free hydrochloric acid into the duodenum is a direct stimulant to the excretion of pancreatic juice. Bayliss and Starling believe, however, that the stimulus to the pancreatic secretion is not the free acid but is a ferment, secretin,⁴ which is formed by the action of the hydrochloric acid upon the intestinal mucosa. While the trypsin acts upon protein bodies, splitting them through various stages into the ultimate products, amino-acids and hexone bases, there is a second ferment, erepsin, discovered in the intestinal mucosa by Cohnheim, which acts upon the intermediate splitting products of protein, such as the albumoses and pepton, carrying this conversion to the same lengths as does trypsin.⁵ It is interesting to note that we find in the intestinal juice of the infant a ferment, lactase, which hydrolyzes lactose into the simpler saccharids.

The presence of the bile, which reaches the duodenum through the common duct, facilitates the proper digestion and absorption of the fatty substances of the food.⁶ Variations in this constituent may reflexly cause

¹ Meltzer, *Am. Jour. Med. Sc.*, 1918, CLIII, 469; Lyón, *Jour. Am. Med. Assoc.*, 1919, LXXIII, 980. Brown, *Ibid.*, 1920, LXXV, 1414. Cook and Wilhelm, *Missouri. State Med. Assoc., Jour.*, 1921, XVIII, 230; Bassler, Luckett & Lutz, *Am. Jour. Med. Sc.*, 1921, CLXII, 674.

² *Deutsch. med. Wehnschr.*, 1909, XXXV, 706. See Izar, *Policlinico*, 1916, XXIII, 741; Krieger, *Arch. f. Verd.-Kr.*, 1920, XXVI, 351; Schoppe, *Ibid.*, 1921, XXVIII, 280; McClure, Wetmore & Reynolds, *Arch. Int. Med.*, 1921, XXVII, 706. Kai, *Jour. Biol. Chem.*, 1922, LII, 133. Strauss, *Med. Klin.*, 1921, XVII, 1577; Northrop, *Jour. Gen. Physiol.*, 1922, IV, 227, 245, and 261.

³ Long and Fenger, *Jour. Am. Chem. Soc.*, 1915, XXXVII, 2213; *Ibid.*, 1916, XXXVIII, 1114; Long, Hull and Atkinson, *Ibid.*, 1915, XXXVII, 2427; Long and Hull, *Ibid.*, 1916, XXXVIII, 1020; *Ibid.*, 1917, XXXIX, 162; *Ibid.*, 1921, 1051; Fenger and Hull, *Jour. Biol. Chem.*, 1919, XXXVIII, 487; Wago, *Arch. Int. Med.*, 1919, XXIII, 33 and 251.

⁴ See Carlson, Lebensohn and Pearlman, *Jour. A. M. A.*, 1916, LXVI, 178.

⁵ See Schlecht and Wittmund, *Deutsch. Arch. f. klin. Med.*, 1912, CVI, 517; Friedmann, *Med. Record*, 1912, LXXXI, 355; Abderhalden, *Ztschr. f. physiol. Chem.*, 1912, LXXVIII, 344.

⁶ See Holmes, *Am. Jour. Dis. Child.*, 1916, XI, 405; Hutchison and Fleming, *Glasgow Med. Jour.*, 1920, XCIV, 65.

disorders in the secretion of the stomach, one finding very frequently hyperacidity associated with obstructive jaundice. Whether the bile may be assumed to have a disinfecting power must be left for the future.

Estimation of Intestinal Digestion.

A careful chemical examination of the feces, coupled with a macroscopic and microscopic investigation, will give much information regarding the degree of intestinal digestion and absorption. However, such methods are time-consuming and not easily performed by the general practitioner. For this reason Sahli has introduced a method, similar to his stomach method, of investigating such activity. He employs glutoid capsules, which are made from gelatin hardened with formaldehyd. These capsules either do not dissolve in the gastric juice at all or only after considerable time, although they are quickly soluble in the intestinal juice. In these capsules is placed material which will not diffuse through the capsule wall and whose absorption may be studied from an examination of the saliva or urine. Sahli uses either iodoform or salol. In the former case 0.15 gram of iodoform is placed in a glutoid capsule, and given with an Ewald test meal. Under the best conditions (normal gastric motility, normal intestinal digestion, and normal intestinal absorption) the iodine reaction may be expected to appear, according to Sahli, in the saliva within four to six hours; that is, within one and one-fourth hours after the capsule has been dissolved by the pancreatic juice. Instead of iodoform salol may be used, being given in the amount of one-half gram along with an Ewald test meal. The reaction for salicylic acid may be obtained in the urine within one and one-half hours after the capsule has been taken.

Sahli gives the following results obtained by the use of this capsule: When the stomach contains neither free hydrochloric acid nor pepsin, the reaction is not delayed so long as gastric motility is good. In cases of diarrhea due only to an increased peristalsis without any marked disturbance of intestinal digestion, the reaction is either normal or even somewhat hastened. In other types of diarrhea characterized by an involvement of the intestinal chemistry or intestinal absorption, the reaction is either absent or distinctly delayed or the capsules may be found undigested in the feces. This method also aids in differentiating an icterus due to occlusion of the ductus choledochus at its point of entrance into the intestine, in which case the digestion of the capsule may be interfered with, from one where the obstruction to the bile flow is higher up near the liver. This test may be of some presumptive evidence in the diagnosis of pancreatic carcinoma, although all cases of pancreatic carcinoma do not necessarily occlude the duct, in which case a positive result would obtain. Galli reports a case of carcinoma of the pancreas in which the glutoid capsule was dissolved, although no pancreatic juice was present in the bowel.

II. MACROSCOPIC EXAMINATION

(1) Method.

The macroscopic examination of the feces embraces not only the study of the physical characteristics, but also a recognition of various normal and

abnormal substances.¹ It is convenient, when examining the feces, to employ some form of a washing apparatus to separate the coarser from the finer particles.

Boas has introduced a special sieve for such work which, however, does not have much advantage over the ordinary flour-sifter which Einhorn advises.

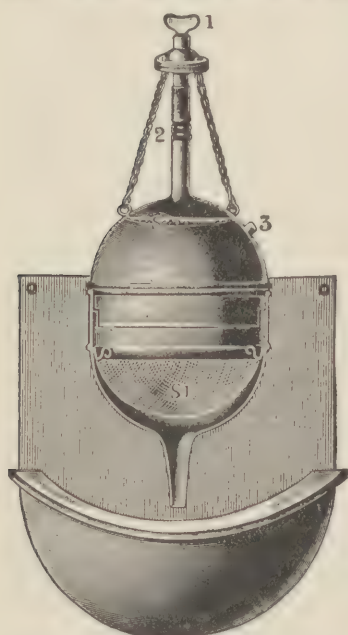


FIG. 20.—Boas' stool-sieve.
(Hemmeter.)

Strauss' method of washing the feces by a current coming from below seems to be much the best of any of these types of apparatus. In the absence of any other equipment, an ordinary household sieve of various-sized mesh will answer the purposes. Small amounts of mucus or connective tissue fragments may be recognized by rubbing up a portion of the stool in a mortar with a little water, when these substances will float upon the surface. In some cases it may even be advisable to place the feces in tall glass jars in which the stool is mixed with water and allowed to arrange itself in layers.

If the stool is of ordinary consistency, it should be spread out in a more or less thin layer so that the larger particles may be easily recognized. If it be, however, a watery stool the contents are thoroughly mixed and examined as such.

(2) Amount.

The amount of feces excreted will depend upon (1) the quantity and quality of the food; (2) the remnants of intestinal juices and débris; (3) the condition of the digestive organs, and (4) the bacteria. These factors are all important, each being dependent to a certain extent upon the other.

The average moist weight may be considered as varying from 100 to 250 grams with a dry weight of 20 to 40 grams. This amount may, however, reach as high as 20 kilograms as in a case reported by Lynch.

Number of Stools.

The number of stools which may be passed in 24 hours is subject to very wide variation, even under physiological conditions, but is usually constant in the same individual. At least one stool a day should be considered normal, although many persons are accustomed to have only one movement of the bowels in 48 hours and others in longer periods. We must, therefore, consider each individual case before judging as to abnormality in the number of stools. Lynch² has reported a case of a patient having only one stool each 100 days, while Geib and Jones³ discuss a case in which there was no stool during an entire year the patient at the end of that time voiding 32 liters of feces.

¹ See Grover, *Jour. Am. Med. Assoc.*, 1921, LXXVI, 365.

² Thesis, Buenos Aires, 1896.

³ *Jour. Am. Med. Assn.*, 1902, XXXVIII, 1304.

A diarrhea is said to exist when the stools are frequent and fluid. The frequency may vary from 2 to 50 in 24 hours, although a single liquid stool in 24 hours may constitute a diarrhea in a patient unaccustomed to having a movement every day. A normal stool is never fluid, so that the character of the stool is of more importance than the number, as individual peculiarities must be considered. A diarrhea may be due to increased peristalsis, to increased intestinal secretion, a diminished gastric secretion, or a decreased absorption from the bowel. The most extreme grades of diarrhea are observed in Asiatic cholera, dysentery and the summer diarrhea of infants, although a marked diarrhea does occur in enteritis, peritonitis, intestinal tuberculosis, and uremia. Some infectious diseases are more frequently associated with diarrhea than are others, but little of diagnostic importance is obtained from this symptom.

By constipation is meant the infrequent and irregular movement of the bowels, associated with symptoms which are relieved by administration of laxatives. The habits of the individual must be taken into consideration before judging as to a real or apparent constipation. This condition is physiologically a result of a sedentary life or of a diet lacking in elements which will stimulate intestinal peristalsis.¹ Pathologically, we find constipation in cases of dilated stomach, occlusion of the bowel, atony of the bowel wall, in conditions causing increased cerebral pressure, and in obstruction by pressure from without the bowel. It must be stated that we may have an apparent diarrhea as a symptom of constipation high up in the bowel. It is better practice to administer a laxative in such conditions than it is to give an astringent.

(3) Consistency and Form.

The consistency and form of the normal stool vary considerably depending upon the nature of the food ingested. The stool is much softer with a purely vegetable diet, of which about 85 per cent. is water, than with an animal diet, of which only 65 per cent. is water. One differentiates, as regards consistency, a well-formed, a mushy, and a fluid stool, between which types there are many gradations, some stools being partly formed and partly fluid. Many factors may influence the amount of water present in the stool, such as (1) a lessened absorption of water from the intestinal canal; (2) intake of a large amount of water, and (3) an increased secretion from the intestinal glands.

The consistency of the normal stool varies from day to day and can be constant only when the patient is placed upon a standard diet. It may be abnormally too fluid or too solid, in the latter case being frequently voided in the form of very hard masses, known as "scybalæ." As a general rule, it may be stated that the greater the absorption of water from the intestine the more firm will the feces be and, in consequence, the more frequently will these scybalæ form. Stools are frequently observed of which the consistency is normal but of which the size of the cylinder is quite small. Such small "lead-pencil" sized stools have been supposed to be indicative of stricture of the lower bowel, but this condition is not necessarily present.

¹ See Cannon, Jour. Am. Med. Assn., 1912, LIX, 1. See Mellanby, Quart. Jour. Med., 1916, IX, 165.

Besides the water-content of the feces, the amount of fat, mucus, and vegetable residue has much to do with the consistency of the stool. One may differentiate the fat from water in the stool by placing a small portion of the feces upon a slide and pressing a cover-slip down upon it. If the cover-glass remains when the pressure is removed increased fat may be assumed, while if the softness of the feces be due to increased water the cover-glass will spring away from the feces.

In some cases, especially those associated with achylia gastrica, the stools are very frothy, indicating a marked bacterial decomposition. Such stools should not be confused with those of the ordinary diarrhea or with the characteristic "rice-water" stools of Asiatic cholera, in which particles of mucus are readily detected. Such stools are never found associated with large fat contents.

(4) Odor.

The peculiar odor of the normal stool is referable to the presence of indol and skatol, which arise from the putrefaction of protein material in the large intestine. Along with these we may find other substances, such as hydrogen sulphid, methane, and phosphine. The odor of the stool is much more marked following a meat diet than that after a vegetable diet; it is very slight on a milk diet and is practically lost in the fasting condition. If the processes in the intestine are of such a nature that fermentation of the carbohydrates exceeds the putrefaction of the proteins, the stool shows a distinct sour odor traceable to the presence of butyric or acetic acid. The odor in cases of acute and chronic diarrhea is frequently very slight, while that of the loose, watery discharges of cholera is peculiar and sperm-like, referable to the presence of cadaverin. In the diarrhea of children a distinct putrid odor may be present, although this is not necessarily the case. The so-called acholic stools have in themselves, according to Schmidt, very little odor, showing this property only when complicated by decomposition processes arising from the lack of bile. In cases of severe dysentery and carcinoma of the large intestine an intensely disagreeable odor is observed which differentiates these conditions from those associated with other types of decomposition.

(5) Color.

The color of the stool varies from a light brown to even a black, depending upon the kind of food, the residue of the intestinal secretions, the presence of pathologic products from the intestinal wall, and the administration of therapeutic agents. The dark color of the normal stool is due to the presence of hydrobilirubin, which is formed from the secreted bilirubin by the reducing processes in the intestines. Bilirubin is found normally in the stool of a nursing child, being converted either into biliverdin or hydrobilirubin under abnormal conditions. This change from a light yellow infantile stool to a greenish one has much importance to the pediatrician. A well-formed stool is always darker in color than is the thin stool, which is equivalent to saying that the stool is darker the longer it remains in the intestine. The color of a stool under a meat diet is dark brown, with a vegetable diet a lighter brown, and following a milk diet a light yellow. This color which is traceable to the

diet is shown best in those cases in which bile is excluded from the bowel. The dark color of the meat feces is probably traceable to the conversion of the blood-coloring matter into hematin and not to the formation of sulphid of iron which is so commonly stated. Food products may color the feces a characteristic shade. Thus, coffee may give a dark brown color, cocoa a brownish-red color, red wine a smoky black-brown color which has a shade of green. Chlorophyll-containing plants, such as spinach and lettuce, give rise to greenish shades.

Occasionally the reduction process in the intestines may go as far as to convert the bilirubin into leucohydrobilirubin instead of into hydrobilirubin. This hydrobilirubin is identical with the urobilin found in the urine. Such stools may be practically colorless when voided, but will be converted into the dark brown normal-colored stool on contact with the air. Besides these pigments, biliprasin has been isolated from the feces by Fleischer, while Müller obtained cholecyanin.

The variations in color of the stool following administration of therapeutic agents is frequently characteristic. Thus, after the use of calomel one observes a distinct green coloration due to the conversion of the bilirubin into biliverdin. Bismuth preparations color the stool a distinct black, due to the formation of the oxid or sulphid of bismuth. Rhubarb, senna, santonin, and gamboge cause a distinctly yellow coloration which will change to a reddish tone in the presence of alkali. Iron compounds produce shades ranging from dark brown to black while methylene blue gives rise to the production of a blue-green color and sandal-wood a reddish-violet color.

Pathologic Variations in Color.

Cases showing the presence of large amounts of mucus or of pus in the feces are characterized by a gray-white or yellowish-gray coloration of the feces. Such cases are seen in membranous colitis and rupture of an abscess, especially of the appendiceal variety, into the intestinal tract. In cases of syphilitic or carcinomatous ulceration of the colon or rectum this characteristic color tone may be more or less influenced by the presence of blood.

Stools showing the presence of a large amount of fat are clay colored. That this coloration is due to the excess of fat, rather than to the absence of bile, may be shown by extracting the feces with alcohol and ether, in which extraction the bile will be taken up along with the fat and will color the solvents. These acholic stools as they have been called occur both in cases associated with biliary obstruction and in those showing no obstruction. It would be better practice, therefore, to style these stools colorless, instead of acholic stools. Strümpell was able to obtain stools of a light brown color by feeding patients a diet containing small amounts of fat, thus proving that the increased fat was more important than the diminution of bile, although this latter does account for some cases. This clay-colored stool may also be found in diarrhea, while in Asiatic cholera and dysentery the stools may be absolutely colorless. They have been also found in cases of leukemia, carcinoma of the stomach or intestine, tuberculous enteritis, and chronic tuberculous peritonitis. The cause of this lack of color may be the same unknown cause that produces the formation of leucohydrobilirubin spoken of above.

In some cases we find a distinct golden-yellow or even a green color of the feces.¹ This is due to the presence of unaltered bile, on the one hand, and biliverdin, on the other. Biliverdin is usually found in abnormal decomposition processes in the intestine of the infant, while unaltered bile may appear in cases of increased intestinal peristalsis, in which the contents of the bowel are rushed onward before absorption can take place. This is probably the partial explanation of the green stool following the administration of calomel. Normally, bilirubin is not found in the intestine below the ascending colon. Such being the case, it may be possible to judge of the point of irritation in a diarrheal attack by the fact that the higher up in the bowel the point of disturbance the more of this pigment will be found in the feces. Bilirubin indicates, therefore, an enteritis especially of the small, but also of the large intestine. This bile pigment may be found most frequently on cellulose material, mucus, muscle fibers, and fat. It is readily detected by rubbing up 2 or 3 c.c. of the feces with a concentrated aqueous solution of pure mercuric chlorid. This mixture is allowed to stand 24 hours and is examined microscopically thereafter. The fragments to which the pigment is attached will stain red if due to hydrobilirubin, while those stained with bilirubin will show a green color. Naturally, in this examination chlorophyll-containing fragments must be excluded. The most favorable material for such examination is the mucus. A green color of the stools may also be observed due to infection with the *bacillus pyocyaneus*.

Blood may give rise either to a distinct red color, a brownish-red shade, or even a black tint. If the blood be adherent to the scybalous masses or to the well-formed feces, it is usually derived from the rectum or anus and indicates hemorrhoids; if it be evenly distributed with the food material and is changed from a bright red to a brownish color, it indicates a hemorrhage in the stomach or high up in the small intestine, especially if the stool be solid. While an evenly mixed bloody feces of fluid character will usually point to the colon as the seat of the trouble. As a general rule, it may be said that the darker the color the more remote from the rectum will be the hemorrhage; Tarry black blood is seldom of low origin, usually indicating trouble in the stomach or duodenum, while fluid scarlet blood usually arises from the colon or rectum, although in some cases of typhoid fever the blood may be a bright red, although the hemorrhage may be fairly well up in the bowel. In cases of intussusception the blood may appear mixed with serum, but with no fecal matter.

In deciding as to the importance of blood in the feces one must naturally exclude that arising from food or from hemorrhages above the gastro-intestinal tract. Thus blood coming from the mouth, nose, throat, or lungs may pass into the stomach and out with the feces, making a mistake in diagnosis very possible. Further, blood arising from vaginal discharges, which may be mixed with the feces at the time of defecation, should be excluded.

The detection of blood in the feces is more or less simple and direct in the fresh state, but when intimately mixed with the feces its recognition is a

¹ See Freudenberg, *Berl. klin. Wchnschr.*, 1922, I, 21.

matter of some difficulty. In many cases of hidden or occult bleeding blood is never detected microscopically or macroscopically. It is practically useless to search for blood-cells in the feces, as rarely are perfect cells found, unless the blood is present in very large amount, many specimens showing no cells.

Guaiac Test (Van Deen's Test).

A small portion of the stool is rubbed up with water and one-third of its volume of glacial acetic acid added. This mixture is well shaken in a test-tube and a few cubic centimeters of ether added. After thoroughly shaking this mixture, it is allowed to settle, when the ether, in the presence of blood, will have assumed a brownish color. In case the ethereal extract is not clear, a few drops of alcohol may be added. On adding to this ethereal extract a mixture consisting of equal parts of fresh tincture of guaiac¹ and ozonized turpentine, a blue ring will form at the point of contact or a blue coloration will be seen throughout the mixture if the tube be shaken.

This test is much more reliable in its negative phase than in its positive phase. The writer has frequently found positive tests for blood arising from the employment of tubes previously used with copper solutions or with nitric acid, so that he would advise the worker invariably to use either new or thoroughly clean test-tubes when testing for the presence of blood. Moreover, this test is given by other substances, which may react positively. If the patient eats watermelon, prunes, potatoes or rice, has been taking iron, or the feces contain much pus, a distinct reaction may be present.² In this test as well as in all of the other tests outlined, the presence of hematin arising from the meat of the diet must be excluded. This can be done only by placing the patient upon an absolutely meat-free diet for several days preceding the examination.

The Schaer-Klinge Test.

This test is very similar to the preceding, but is much more delicate, being positive after the ingestion of only three grams of blood. It is even more important when using this test to exclude all hemoglobin and chlorophyll-containing foods for some days preceding the examination. The stool is rubbed up with water and treated as in the preceding test with acetic acid and ether. To this ethereal extract is then added a mixture of 1 c.c. of ozonized turpentine and $\frac{1}{2}$ c.c. of fresh 3 per cent. alcoholic aloin solution. This may be prepared by dissolving what aloin will lie on the point of a spatula in $\frac{1}{3}$ of a test-tube of 95 per cent. alcohol. At the line of contact a distinct red ring will be observed in the presence of blood in from three to five minutes. Careful work with this test has shown that fat interferes to some extent with its delicacy. It is, therefore, customary to treat the feces with an equal volume of ether and to shake thoroughly to remove all the fat present. The ethereal solution is then poured off and the remaining fecal material mixed with one-

¹ Lyle and Curtman, *Jour. Biol. Chem.*, 1918, XXXIII, 1, advise the use of guaiaconic acid. See Boas, *Berl. klin. Wchnschr.*, 1916, LIII, 1357; Lemay, *Jour. pharm. Chim.*, 1920, XXII, 52.

² Boiling the watery suspension of the feces and allowing it to cool before adding the acid and ether will prevent reactions arising from ferments and oxygen transmitters other than blood; Rapin (*Rev. Méd. de la Suisse Rom.*, 1916, XXXVI, 235) shows that charcoal, given to mark the feces, may act as a catalyser in various tests for occult blood.

third its volume of glacial acetic acid and 10 c.c. of ether, being then thoroughly shaken and set aside.

A portion of this brownish ethereal extract, which contains the hematin formed by the action of the glacial acetic acid upon the hemoglobin of the blood, is poured into a thoroughly clean test-tube and treated as above described with the turpentine and aloin solution. If the tube be shaken after the red contact ring has formed the whole mixture will assume a cherry-red color. If the tube is allowed to stand for a few minutes the aloin solution may sink to the bottom, forming a distinct red layer beneath that of the ether and turpentine. A reaction to be positive should appear within 10 minutes as the aloin itself will gradually turn red under the conditions of the experiment if left for a much longer period. Charcoal instead of carmin should be used to mark the feces. The writer has found this test very reliable and very easy of application.

Weber's Test.

This test has the advantage of excluding practically every other factor which might influence the tests previously given.¹ A portion of the feces is extracted with ether to remove the fat and is then separated from the ethereal solution. This fat-free feces is then rubbed up with water and treated with glacial acetic acid and ether. The ether, as in the preceding test, takes up the hematin which is now detected by the spectroscope, showing the characteristic spectrum, namely, an intense narrow band in the red between C and D and a somewhat more definitely marked group of three broader bands, (1) in the yellow, (2) at the boundary between yellow and green, and (3) at the boundary between the green and blue, this last band being difficultly recognizable.

In order to avoid confusion with the spectrum of methemoglobin or of chlorophyll, one may convert the hematin into reduced hematin (hemochromogen) by the addition of alcoholic potassium hydrate, water, and ammonium sulphid solution. The spectrum of this latter substance is characterized by the two bands in the green.

This test is probably the most sensitive and should be more frequently employed. It is very simple of application, is very reliable and has practically no fallacy, especially when the hematin is converted into the hemochromogen. This test is, however, not so delicate as the aloin test.

Weber originally used the guaiac test with a modification of extracting the fat from the feces before testing with guaiac and turpentine. Schumm states that if the feces be thoroughly extracted with alcohol and ether most of the fat and urobilin will be removed and that under these circumstances Weber's test, either with the guaiac tincture and turpentine or with the spectroscope, becomes much more valuable.

¹ Queisser (Therap. Monatsh., 1913, XXVII, 727) found blood by this test in cases fed upon the Lenhartz gastric ulcer diet, such cases being free from gastro-intestinal ulceration. Snapper (Arch. f. Verd.-Kr., 1919, XXV, 230) extract the feces with acetone and then with acetic ether. He then shakes out the porphyrin from the ethereal extract with HCl and examines spectroscopically. See, in this connection, Boas, *Ibid.*, 1920, XXVI, 37; Snapper and Dalmeier, *Deutsch. med. Wchnschr.*, 1921, XLVII, 985; Koopman, *Arch. f. Verd.-Kr.*, 1921, XXVII, 122. Van Eck, *Pharm. Weckblad*, 1921, LVIII, 723.

Adler's Test.

O. and R. Adler have introduced the use of benzidin as a test for the presence of blood. The stool is extracted with a mixture of alcohol and ether for the reasons above mentioned. It is then treated with glacial acetic acid and with ether as described in the other tests. This acid ethereal extract, which contains the hematin, is then treated with 2 c.c. of a saturated alcoholic benzidin solution and 2 c.c. of hydrogen peroxid (3 per cent.). In the presence of blood a greenish-blue color appears.

Wagner¹ advocates a so-called "dry test" of the stool which is delicate, reliable and extremely simple. He uses as reagent the Schlesinger-Holst mixture of a knife-point of benzidin, 2 c.c. of glacial acetic acid and 20 drops of a 3 per cent. solution of hydrogen peroxid. If a few drops of this mixture be poured over a little of the solid feces spread on a clean glass slide, a greenish-blue color will appear almost immediately in the presence of blood. This benzidin test is very sensitive and can be recommended for general use.

(6) Mucus.

From the diagnostic standpoint the recognition of much mucus in the feces is of the greatest importance. Any amount of visible mucus should be considered pathological, although it is to be remembered that mucus may be increased physiologically as the result of hypersecretion, in which case it will appear as a slimy coating of the scybalous masses or as small adherent particles. Boas regards the mucus found after strong cathartics as normal, but this is to be questioned as the irritation may be sufficient to set up a mild hypersecretion. The mucus expelled with the meconium and, according to Lynch, even that passed by infants up to the second week of life should be considered normal. The fecal mucus is a true mucin, being precipitated by acetic acid, but dissolving in 10 per cent. HCl.

The larger portions of mucus may be easily recognized with the naked eye, but the smaller bits are more clearly brought out by rubbing up the feces with water and holding the material, in a thin layer, toward the light. If the feces be well formed, the mucus may be separated from the exterior of the cylinder, as it is never found in the interior of a firm feces. In mushy stools the mucus is intimately mixed with the fecal material, usually in the form of smaller particles, the exterior type of mucus being of much larger flakes. Nothnagel has reported a jelly-like consistency of a mushy stool in a case of jejunal diarrhea, in which the mucus was not derived from the intestinal wall, but came probably from the bile. It is important that one be able to recognize mucus, as mistakes have been made in the presence of swollen vegetable tissue, fruit pulp, echinococcus membranes, and even of parasites.

¹ Zentralbl. f. Chir., 1914, XLI, 1182; Arch. f. Verdauungskr., 1914, XX, 552. See, also, Lyle, Curtman and Marshall, Jour. Biol. Chem., 1914, XIX, 445; Zengerle, Med. Klin., 1914, X, 1795; Indemans, Nederl. Tijdschr. v. Geneesk., 1915, LIX, 2077; Roberts, Jour. Am. Med. Assn., 1915, LXV, 244; Couturier, Lyon Méd., 1914, XLVI, 313, advocates the use of hematin as a reagent for occult blood; Gregersen, Ugesk. f. Laeger, 1916, LXXVIII, 1266; Kelly, Jour. Lab. and Clin. Med., 1916, I, 897; Vaughan, Ibid., 1917, II, 437; Weehuizen, Chem. Weekblad., 1918, XV, 1521; Graham, Jour. Med. Res., 1918, XXXIX, 15. Gregersen, Arch. f. Verd.-Kr., 1919, XXV, 169; Schlesinger and Gattner, Berl. klin. Wchnschr., 1919, LVI, 706; Boas, Ibid., 939; Van Eck, Pharm. Weekblad, 1920, LVII, 219; Penn, Med. Jour. Australia, 1920, I, 525; Menten, Brit. Jour. Exp. Path., 1920, I, 235; Adler, Arch. f. Verd.-Kr., 1921, XXVII, 153; Pron Arch. de Mal. de l' App. Dig., 1922, XII, 204.

The ordinary form in which mucus appears in the feces is in clumps, flocculi, or shreds with irregular margins. These pieces may vary in size from those just visible to those several inches in length. In some cases strips, tubes, ribbons, or macaroni-like pieces are observed, which are especially frequent in enteritis membranacea or mucous colitis. The amount of mucus passed may vary from a few flakes to an enormous mass, Bories having seen 120 grams in one movement. Occasionally one may see, especially in the stools of infants, masses resembling cooked sago granules¹ or "frog spawn" which Kitagawa has identified as mucinous material.

The consistency of fecal mucus varies from that of a jelly-like mass to one having the density of thin leather. The larger the piece the firmer it appears, although exceptions do occur. Pure mucus is usually glassy or jelly-like, certain inclusions changing its consistency. Cellular inclusions in the mucus change it to a paper-like mass, while some specimens appear tenacious due to absorption of protein material or to a diminution of its water-content. These inclusions, as well as those of fat or bacteria, will diminish the usual transparency of the mucus.

On remaining long in the bowel, mucus may take on the normal brown color of the feces due to hydrobilirubin, a dark orange shade from bilirubin, a greenish hue from biliverdin, or a red to reddish-brown tinge from blood pigments. The usual state of the mucus, however, is colorless.

It may be stated generally that the most of the macroscopically recognizable mucus of the feces arises from the large intestine. Owing to its easy digestibility the mucus from the mouth, stomach, and upper intestines passes out only under conditions of great motility. The secretion of mucus in the small bowel is much less than in the large intestine. Of all recognizable forms of fecal mucus only the smallest particles arise from the small bowel, and then only when they are found in a fluid feces. The higher the secreting point the smaller will the particles be, as a rule. These upper intestinal flecks contain much detritus of digestion and half-digested cells or free nuclei and crystals in cellular form, frequently a few bilirubin granules being observed.

The mucus from the large intestine, especially from the sigmoid, is large in amount, is jelly-like in appearance and has ordinarily no inclusions. In cases of mucous colitis the mucus may be passed in the forms of the large strips or bowel-casts, appearing as pure transparent material grayish-white or bloody in color and having no inclusions. No fecal matter may be present. Such a condition is a pure secretion neurosis and becomes a true inflammation only when cell-inclusions are observed. It is to be said that mucus, even with a small inclusion of pus or blood, does not necessarily point to ulceration. On the other hand, one must not judge from the absence of mucus that no catarrh exists, as one frequently finds variations in such excretions.

Microscopic mucus is much rarer than the macroscopic type, although both may be present at the same time. Microscopically, mucus appears as a structureless mass, characterized by irregular lines running through it and by a difficultly recognizable margin, showing a more or less transparent ground

¹ Ullmann, *Med. Klinik*, 1916, XII, 1230.

substance in which may or may not be found epithelial cells, pus-cells, blood-cells, bacteria, protozoa, food remnants, and crystals. If the mucus comes from the higher sections of the bowel the food remnants will predominate, while in that from the lower bowel the cellular elements are in excess.

Mucus is stained with difficulty. For a successful stain the reaction must be neutral and very little admixture with foreign material is permissible. Thionin colors mucin a specific violet, while the other tissue elements are stained blue. Methylene blue and methyl violet stain it but slightly, while other aniline dyes color only the enclosed cells. Iodin may give a diffuse yellow.

(7) Pus.

Macroscopically visible masses of pus of a gray-white color are occasionally found in the stools, but these may hardly be distinguished from mucus particles without the microscope. If in large amounts, attention should be directed to the perforation of an abscess into the bowel. Such pus is more or less intimately mixed with the fecal material. The passage of pus from the small intestine and even from the cecum is associated with such a marked decomposition that it can be recognized only with the greatest difficulty. The nuclei may still persist, but these resemble very closely those of the food cells.

A few isolated leucocytes are usually present in the feces, as a result of diapedesis through the mucous membrane. A pure pus excretion is never seen in cases of uncomplicated catarrh, but in ulcerative processes of the large intestine and in many affections of the small bowel (dysentery, ulcerative colitis, syphilis, carcinoma, tuberculosis, and typhoid) pus-cells may be found in small masses.

Casein flocculi should be differentiated from pus masses by microscopic examination, which will show fat droplets mixed with the albumin.

(8) Food Remnants.

The appearance of macroscopic amounts of food remnants in the feces is known as *lientery* and is dependent upon the nature and amount of food, its method of preparation, the degree of its mastication, and upon the condition of the digestive organs.

Much less undigested material remains on a meat diet than after a vegetable ration. In the former case we may find small bits of bone, cartilage, tendon, hair, feathers, skin, fish scales, and connective tissue; while in the latter we may observe cellulose-containing cells, such as those of cereal, cotyledonous and leguminous vegetables, skins of fruits, nuts, and vegetables, seeds, etc.

Cooking has a great influence upon the digestibility of any food. Boiling seems to be the best method, although much of the nutritive material is taken up by the cooking water. The method of roasting is such as to produce a more nutritive but a less digestible substance, unless it is carried only to the point of slight coagulation of the protein. The outside layers of roasted meats seem to be practically indigestible, according to the work of van Ledden Hulsebosch. Smoking seems to be the least desirable form of preparing meats. Vegetables become more digestible through the cooking process, owing to the bursting of the indigestible cellulose membrane. Those vege-

tables, such as lettuce, cucumbers, onions, turnips, cabbage, and radishes, which are eaten raw, appear in the feces absolutely unchanged.

While great individual differences exist in the power of utilizing digestible or undigestible food, yet we must assume that any appreciable residue, especially following the Schmidt-Strasburger diet, is pathological. The digestive insufficiency may begin in the mouth as a result of too little mastication. The importance of thorough mastication has been especially emphasized by Fletcher.¹ Lack of digestive power of the stomach will have but little influence on intestinal digestion, providing the pancreatic secretion is sufficiently active, with the exception that raw or smoked connective tissue will be undigested and will appear in the feces. Lientery is much less frequent in motor disturbances of the stomach than in those of the intestines. Perhaps the greatest amount of undigested residue is seen in cases of perforation of an ulcer or carcinoma of the stomach into the intestine, a direct communication being established between the stomach and colon.

Naturally, in cases of increased intestinal motility, one will find more food residue in the feces than under normal conditions. This motor insufficiency is especially answerable for the appearance of undigested starch remnants, as, even under the most severe conditions, starch digestion is not interfered with to any extent in the bowel, providing mastication and preparation of food have been sufficient and the succus entericus has not been so diminished by catarrhal processes as to permit of fermentation. The direct irritation from the hard particles of cellulose may cause an increased peristalsis and, hence, directly lead to lientery.

Marked disturbance of protein and fat digestion, evidenced by an intense lientery, will occur if the pancreatic secretion be insufficient. This is even more marked if gastric disturbance coexists. A lack of bile will cause an exclusive disturbance of fat digestion, which is seldom manifested by the appearance of macroscopic particles of fat, but is characterized by the typical clay color of the stool.

The final factor which influences lientery is the insufficiency of the absorptive power of the intestine. This increases the food residue by retarding digestion, according to the law that an accumulation of the products of ferment activity will prevent further action of the ferment, as well as by holding within the bowel the products already digested.

Only in exceptional cases can one judge of the specific factor at the bottom of a lientery, a boundary line between normal and abnormal being drawn with difficulty. It is, however, important that one be able to recognize food remnants both macroscopically and microscopically. The writer recalls a case in which the residue of orange and banana pulp was mistaken for a new parasite. This recognition of the nature of a fecal residue is especially important in the examination of the infantile stool. Normally, the nursing child shows no food residue in the feces, but frequently white flecks or clumps are observed, which may consist of casein but perhaps more frequently of

¹ See, however, Foster and Hawk, Jour. Am. Chem. Soc., 1915, XXXVII, 1347.

fat or of soaps. The proper recognition of such particles will require both chemical and microscopic examination.

The following discussion of the appearances of the various food residues will include both the macroscopical and microscopical examinations, as these are inseparable in practical work.

(A) Protein Residues.

(a) Muscle Fibers.

The appearance of muscle fibers in increased amount in the stool is known as *azotorrhea*.¹ The muscle fibers appear as isolated pieces of different size and shape. The smallest pieces have a circular or oval contour, the medium-sized particles are jagged, while the larger masses have parallel sides and angular surfaces. These fibers are colored yellow or yellowish-brown by the hydrobilirubin, but may be tinged with bilirubin or by foreign pigments. The color will depend both upon the amount of pigment and upon the time the fibers have lain in the bowel.

The smaller pieces may be entirely homogeneous, although these as well as the larger bits show both transverse and longitudinal striations. If the fibers are well digested, the longitudinal striations may be the only ones showing, and even these may disappear. No nucleus is visible unless the pancreatic secretion is entirely lacking.² No specific micro-chemical reactions are known for these fibers. They may be tested with any of the color reactions for protein material.

Connective tissue and elastic tissue fibers are occasionally associated with muscle fiber and may be recognized by their appearance.

(b) Casein.

This is found especially in the infant stool and is always pathological. These masses, known as curds, are more or less round clumps which vary in size from that of a pin head to that of a hazel-nut. They are either pale or golden-yellow, the larger masses being a pure white inside. These curds always have a distinct yellow tone exteriorly, but the interior is pure white. If these particles are pressed between a slide and cover-glass, they spread out like white cheese and show absolutely no structure microscopically. These masses show the protein reactions.

Leiner's Test.

A small amount of fecal matter containing these curds is spread on a slide and dried in the air. It is then fixed by heat and stained with a mixture of equal parts of a 0.75 per cent. solution of acid fuchsin and methyl green in 50 per cent. alcohol (dilute mixture 10 times with water). At the end of 15 minutes the slides are placed in distilled water and left for one hour. Casein and paracasein will take a pale blue or violet color, while similar substances will show a greenish tone.

Microscopically, one may differentiate from these true casein particles

¹ Also called *creatorrhœa*. See Wertheimer, *Ztschr. f. klin. Med.*, 1912, LXXXVI, 57; Tileston, *Arch. Int. Med.*, 1912, IX, 525; also, Fronzig, *Ztschr. f. klin. Med.*, 1913, LXXXVII, 40.

² See Atchley (*Arch. Int. Med.*, 1915, XV, 654), whose experiments tend to disprove the value of Schmidt's nuclear test.

(curds), certain products which are more or less normal in the stools of the child. These masses are smaller than the curds, being not usually over pin-head size, are more yellow in color, and appear under the microscope as clumps of fatty acid crystals or of fat droplets and bacteria bound together by mucus.¹

(B) Fat Residues.

Fatty substances are present in all stools, if there be any in the diet, either in the form of neutral fat, free fatty acids, or soap, especially of calcium and magnesium. If present in macroscopically recognizable amounts, the condition is known as *steatorrhea*.²

Neutral fat may be present in the form of white colorless clumps of different size and irregular in shape, some being globular while others are distinctly angular. The more usual form is the refractive, opaque, more or less yellow globule. Occasionally the fat may appear as a melted oil which hardens over the surface of a formed stool or gives the appearance of vaselin to a semi-solid stool. Neutral fat may be recognized by its ready solubility in ether and its black color on treatment with osmic acid and deep red color when acted upon by Sudan III or Scharlach R.

Fatty acids appear either as scales or as crystals of varying form. The scales may not be distinguishable from those of pure fat except by their easy solubility in cold alcohol. The crystals are thin, delicate, curved needles, which run to a distinct point and are grouped in thick masses. Other types of fatty acid needles may occur, such as the small lancet-shaped plate or those closely resembling the soap crystal. These crystals are colorless and are soluble in cold alcohol, showing no stain with the above reagents for free fat. The fatty acid scales are, however, stained by these dyes.

The soaps likewise appear in the feces as scales or as crystals. The scales are less refractive, more firm, usually more angular, and may be colorless or yellow-brown from hydrobilirubin or yellow from bilirubin. The crystals appear most frequently as uncolored needles, which are shorter, plumper, not so pointed as the fatty acid crystals, and are arranged in clusters. Schmidt has described a peculiar form of crystal, which he styles the "cracknel" form, a round type with a sunken center and a raised border. These crystals are insoluble in ether, do not melt on warming, and are uncolored with stains. Treated with acids they form fatty acid crystals.

Any condition which interferes with the proper absorption of fat by the mucosa or lymphatics will lead to *steatorrhea*. This condition may be observed, physiologically, after the ingestion of large amounts of fat, as in the oil treatment of gall-stones, but we are little concerned with such findings. It is seen, pathologically, in cases of atrophy of the mucosa, in amyloid disease of the intestines, tuberculous ulceration, tubercular peritonitis, tabes mesenterica, and even in catarrhal enteritis.

The peculiar, glistening, gray-white, pasty, acholic stool of *steatorrhea* is seen more frequently, however, in cases of biliary obstruction and of pancreatic disease. The fat in these cases of biliary stasis is in the usual form, that is

¹ See Courtney, *Am. Jour. Dis. Child.*, 1912, III, 1.

² Miller and Perkins (*Quart. Jour. Med.*, 1920, XIV, 1) report a case of the congenital type.

three-fourths of the ether extract of the bile-free feces is present as fatty acids and soaps and one-fourth as neutral fat. In cases of acholia 50 to 80 per cent. of the fat will be unabsorbed instead of the normal 5 to 10 per cent.¹

In the fatty stools of pancreatic disease, the fat is largely present as fatty acid, no soap being found. The association of steatorrhea with the absence of decomposition products, few bacteria, and the presence of maltose in the feces is much more indicative of pancreatic disease than is steatorrhea alone (Le Nobel).

(C) Carbohydrate Residues.

Starch may be present, in the normal feces, enclosed within plant cells which have resisted digestion, but well-preserved starch granules are never found normally. A few partially digested or complete colorless granules may indicate no abnormality, but many will point to a hyperacidity or to a disturbance in the small intestine, especially to an insufficiency of the succus entericus leading to the so-called "fermentative dyspepsia" (Schmidt).

Starch may be detected by the addition of Lugol's iodine solution to a small portion of the feces spread upon a slide. A blue coloration will indicate starch, while a red tone will show the presence of erythrodextrin.

The varieties of cellulose-containing substances found in the feces are as numerous as are the types of such food material. These have been discussed previously and cannot be here elaborated. Certain microchemical tests may prove valuable in the identification of such cellulose material.

Cellulose treated with sulphuric acid and then with iodine solution gives a blue color due to the conversion of cellulose into amyloid.

If a solution of zinc chloride be allowed to act upon a suspected mass of cells, which have been previously treated with Gram's iodine solution, a purple coloration will be produced. If a neutral or weakly alkaline solution of Congo-red be added to cellulose, it will be stained a distinct red. Cellulose is soluble only in an ammoniacal solution of cupric oxide, known as Schweitzer's reagent.

(9) Biliary Constituents.

As a rule, the unchanged biliary acids, glycocholic, taurocholic, and cholalic acids, are absorbed from the bowel so that they do not appear in appreciable amounts in the feces. Schmidt believes that cholalic acid is present in slight amounts in all feces and that all the bile acids may be increased in pathologic conditions.

Bile pigments, on the other hand, are always present, chiefly in the form of hydrobilirubin, although bilirubin may occur. In pathologic conditions we may find, beside these two pigments, biliverdin, bilifuscin, bilicyanin, and bilihumin. Stercobilin (urobilin) in the feces is usually an index of blood disintegration.² The tests for these pigments as well as for the free bile acids and their salts will be considered in detail in the section on Urine.³

¹ See von Hoesslin and Kashiwado, *Deutsch. Arch. f. klin. Med.*, 1912, CV, 576; Aviragnet and Dorlencourt, *La Nourison*, 1919, VII, 283.

² See Robertson, *Arch. Int. Med.*, 1915, XV, 1072; *Ibid.*, 1915, XVI, 429.

³ See, however, Jovine, *Gazz. d. osp.*, 1913, XXXIV, 1071. For the chemical composition of human bile consult Czyhlarz, Fuchs and von Fürth, *Biochem. Ztschr.*, 1913, XLIX, 120; also, Rosenbloom, *Jour. Biol. Chem.*, 1913, XIV, 241.

In cases of cholelithiasis gall-stones of varying size may be found in the feces, although not in every case. It is, therefore, of the greatest importance that the feces be carefully examined in suspected cases for the presence of these concretions. This is especially the case whenever a severe, colicky, abdominal pain of doubtful origin exists. The feces should be well mixed with water and passed through a fine sieve which will retain any of the suspected particles. A single examination of the feces is not sufficient in such cases unless the stones be found. In cases of negative findings, the stool should be searched for at least two weeks following a suspected attack. According to Naunyn, only the very firm stones will leave the bowel, the softer ones breaking up into small bits in the bowel.

These gall-stones vary from the size of a pin-head to that of a pigeon's egg and are found as small crumbling masses or as hard stones which either have a jagged surface or a smooth surface with characteristic facets, indicating the presence of many such stones. These stones consist for the most part of cholesterin mixed with the biliary pigment, or of compounds of calcium with the biliary pigments. These calcareous stones may be combinations of the various bile pigments and are always the hard facetted type, while the cholesterin stones are softer and may be colorless or tinged with bile pigment. The nucleus of these stones is usually a mass of organic detritus, in some cases being made up of clumps of bacteria, such as the typhoid bacillus and bacillus coli communis. A gall-stone may be usually recognized from its fractured surface, but frequently it becomes necessary to submit it to chemical examination for purposes of identification.

In this examination for gall-stones the worker must not be deceived by the presence of extraneous substances such as seeds, cherry-stones, fats and soaps of high melting point and of masses of impacted feces. In the treatment of gall-stones by the use of olive oil many of these soft fatty translucent masses appear in the feces which are in no way associated with gall-stones.

(10) Intestinal Sand and Concretions.

By intestinal sand we have reference to the small granules or masses of inorganic salts which appear in the feces. These masses are very small, may be spherical or irregular, are usually hard, usually have a reddish-brown or green color and consist of inorganic compounds mixed with organic detritus, especially with fat and bacteria. While most of this material is made up of ingested substances, we may have in cases of neurasthenia and especially in those associated with mucous colitis the excretion of large numbers of these particles as the result of a true secretory neurosis. Myer and Cook¹ have recently shown that the banana may be a source of this intestinal sand.²

The massing together of this intestinal sand may form intestinal concretions or *enteroliths*. As a rule, however, these intestinal concretions have as a nucleus some foreign body around which calcium and magnesium salts of phosphoric and carbonic acid or ammonium magnesium phosphate have been deposited. These enteroliths are usually hard, heavy, and round, being

¹ Am. Jour. Med. Sc., 1909, CXXXVII, 383.

² Talbot (Jour. Am. Med. Assn., 1913, LXI, 238) shows that the banana is not always the source.

colored, as a rule, brownish.¹ A second form of intestinal calculus, known as *coproliths*, are irregular in shape, usually softer than the enteroliths, and consist of inorganic material, mixed with inspissated feces. Neither of these types of intestinal concretion have any great pathologic significance, although the possibility must be granted that some of them might either form or lodge in the appendix and thus be accountable for an acute appendicitis.

(11) Tissue Fragments.

The examination of feces for tissue fragments is more or less unsatisfactory, owing to the fact that these fragments are difficultly recognizable after being partially digested by the juices of the intestinal canal. It not infrequently happens, however, especially in carcinoma of the lower bowel, that such fragments may be obtained and a diagnosis made possible by microscopic examination. A diagnosis of malignancy would better not be made unless these tissue fragments show typical cellular arrangement, or at least, typical arrangement of the nuclei. The writer has seen a diagnosis of carcinoma made on the basis of a strip of mucus with cell enclosures and would, therefore, warn the worker to be on his guard against the possibility of such an absurd mistake.

III. MICROSCOPIC EXAMINATION

This examination includes the search for many substances which have been described under the macroscopic examination, so that these need not be here considered. There are, however, some elements, both morphological and crystalline, which have not been treated.

For any success whatever in the microscopic examination of the feces, great care must be taken in the selection of the material for examination. This is especially true when searching for parasites or their ova, as well as for the differentiation between various food particles and secretions from the intestinal wall. If the stool be soft and mushy it should be thoroughly mixed by stirring and suspicious particles looked for macroscopically. Fresh specimens as well as stained preparations are then made and examined both with the low and high power. In examining feces for bacteria it is always well to mix the feces with water and centrifuge for a few minutes. The bacteria will remain suspended in this process and the coarser fecal material will be deposited. The fluid is then poured off and mixed with equal parts of alcohol, the mixture being again centrifuged when the organisms will be found in the sediment. This procedure is the one adopted by Strasburger in estimating the proportion of bacteria in the dry stool.

Various microchemical reactions are carried out with isolated fecal material and frequently are of great importance. In this procedure a few drops of reagent are allowed to flow under a cover-glass which covers the specimen. The staining material may be drawn under the cover-glass by placing a piece of ordinary filter-paper on the opposite side of the cover-slip.

If the examination for amebæ is to be made it is essential that the stool

¹ See Coerr, Jour. Am. Med. Assn., 1913, LXI, 2238. Raper (Biochem. Jour. 1921, XV, 49) describes a human enterolith containing choleic acid.

be kept warm, as the characteristic differentiating point of an ameba is its active motility. This can be done by placing the feces in a bottle which is surrounded by warm water until the examination is made. It is wise, also, to make the examination upon a warm stage, although this is not absolutely essential unless extended search has to be made for the parasite. There are no specific stains for the feces, the ordinary Löffler's methylene blue serving very well for a general staining of bacteria, while characteristic stains must be used when searching for special organisms.

Morphological Elements.

Epithelial cells are present in every specimen of feces. These may be squamous in form and are usually found in the mucoid particles of the stool. They are especially present in rectal carcinoma and in ulcerative conditions of the lower bowel. The cylindrical type of epithelium is much the commonest form and is found, also, in association with the mucus. Many well preserved cells may be present if their source is in the lower bowel, but, as a rule, these cells show all types of degeneration. They occur in catarrhal inflammations of the intestinal mucosa and are rarely associated with pus-cells unless ulceration has taken place. If the irritation is in the small bowel these cells are always more or less digested and contain bilirubin particles in cellular arrangement, while if from the lower bowel the cell is usually intact.

The presence of red blood-cells is determined more by the chemical examination than by the microscopical. It may occasionally happen that these cells may be seen, but a good result will depend upon a fortunate selection of the material for examination. A few scattered leucocytes are practically always found in the feces,¹ and assume pathologic importance only when present in large amounts as pus-cells, whose occurrence has been previously discussed.

Crystals.

Besides the crystals of free fatty acids and soaps previously mentioned we find large numbers of other crystals in the feces. Among these we observe neutral phosphates of calcium and of magnesium which appear as wedge-shaped crystals occasionally forming rosettes in the former case while in the latter the crystals are more in the form of rhombic plates. The ammonium magnesium phosphate crystals are practically always present in the feces, appearing either as the typical coffin-lid crystal or as irregular fern-like masses. These triple phosphate crystals were at one time supposed to be characteristic of typhoid stools, but like so many other things their importance has been exaggerated. Various crystals of calcium compounds are observed, such as calcium carbonate, calcium sulphate, and calcium oxalate, along with calcium salts of unknown fatty acids, which have been described as irregular, oval, or circular masses, either fissured or showing concentric striations, and being always bile-stained. The lactate of calcium is seen in the form of radiating needles arranged in sheet-like masses in the stools of children on a milk diet.

¹ Albu and Werzberg (*Ztschr. f. klin. Med.*, 1912, LXXIV, 394) call attention to the frequent presence, in the feces of amebic dysentery, of eosinophile leucocytes and myelocytes. See, also, Barnett, *Arch. Int. Med.*, 1917, XIX, 695; Lynch, *Jour. Lab. and Clin. Med.*, 1917, II, 251.

Cholesterin crystals are found as thin, transparent, rhombic plates with notched corners. These crystals do not always appear in the typical shape, so that they should be tested by the addition of concentrated sulphuric acid when the cholesterin crystals will change from a yellow to a blood-red, violet, green, and finally blue color. The Charcot-Leyden crystal appears in typical form in the feces as a colorless, diamond, double pyramid-shaped crystal. The presence of these crystals is practically characteristic of *helminthiasis*, although they may indicate the presence of any parasite from the least harmful to the most pernicious.¹ Hematoidin crystals occur as reddish-yellow rhombic plates or as groups of needles or amorphous masses in stools showing the presence of blood chemically. These have no special significance and are not found as frequently as are the other blood pigments.

After the use of bismuth preparations we find the oxid of bismuth appearing in the form of black irregular rhombic crystals with notched edges. Charcoal appears in the form of irregular black masses which are larger and not so rhombic as are the bismuth crystals.

The examination of the feces for the various bacteria as well as the parasites and parasitic ova will be discussed in a special section. Various substances appearing in the feces have been mistaken for parasites or their ova.

IV. CHEMICAL EXAMINATION

The chemical examination of the feces would naturally embrace both the qualitative and quantitative estimation of the products of digestion and decomposition as well as the estimation of the undigested portion of the food along with the products derived from the gastrointestinal canal itself. Clinically, such work is rarely carried out and has questionable value from a diagnostic standpoint. However, in following the metabolism in any special case it is essential that the absorbed and unabsorbed portion of the food be known. This applies more particularly to the proximate food principles, protein, fat, and carbohydrate, although in some cases the estimation of the inorganic intake and excretion is of first importance.² The writer cannot attempt in the scope of this work to go into great details regarding the chemical examination of the feces, but must limit himself to a few selected topics.

Reaction.

The normal reaction of the feces does not vary much from a neutral one, although it has a tendency to be slightly alkaline, owing to the presence of the alkaline secretions of the intestinal tract.³ The alkalinity of these secretions is diminished both by the combination of the alkali salt with the digestion products and by the absorption of some of this alkaline material into the blood. This reaction may at times be acid owing to the formation of lactic

¹ Acton (Indian Jour. Med. Res., 1918, VI, 157) and Thomson and Robertson (Jour. Trop. Med. & Hyg., 1921, XXIV, 289) believe these crystals to be especially indicative of amebic infection.

² See Holt, Courtney and Fales, Am. Jour. Dis. Child., 1915, IX, 213; von der Heide, Biochem. Ztschr., 1914, LXV, 363.

³ See Bruce, Jour. Lab. and Clin. Med., 1919, V, 61. Robinson (Jour. Biol Chem., 1922, LII, 445) shows that the normal fecal reaction in healthy adults on mixed diets lies between pH 7 and 7.5.

and butyric acids in the fermentative processes, while an increased alkalinity may be observed in cases of markedly increased ammoniacal decomposition. A pure meat diet gives, as a rule, an alkaline feces, while a pure carbohydrate or fat diet will have a tendency to form an acid feces.

The reaction of the feces under pathologic conditions is of little importance, although in typhoid fever the reaction is somewhat more strongly alkaline than in almost any other condition. So much depends upon the diet as well as upon the condition of the digestive organs that no conclusions at present may be drawn from the reaction of the feces.

Total Solids.

The normal amount of feces passed in 24 hours ranges from 100 to 250 grams, of which about 75 per cent. is water and 25 total solids. Although the determination of the total solids of the feces has little of importance in itself, it is essential that one should know the dry weight of the feces in order that he may properly calculate the amount of the various substances in the dry feces.

In determining the total solids in the feces, the specimen, preferably the 24-hour specimen or each movement separately, is placed in an evaporating dish, covered with a small amount of alcohol, and heated over a water-bath with frequent stirring. Small amounts of alcohol should be added as evaporation proceeds in order to hasten the drying process. It is, moreover, necessary to add a small amount (10 to 15 c.c.) of dilute sulphuric acid and to mix it thoroughly with the feces. This combines with the free ammonia, forming a non-volatile salt, and thus prevents loss of nitrogen. If the stool be rich in fats, it is wise to add a weighed amount of dry washed sand to make the mass more porous and thus permit of quicker drying. When the water has been driven off on the water-bath, the specimen may be placed in the drying oven at 105° and left for several hours, after which it is placed in the desiccator and dried to constant weight. Knowing the weight of original substance taken and the weight of the dry substance, it is very easy to determine the percentage of total solids.

An increased total solids of the feces is observed in most cases of constipation, while in diarrhea the solid residue is much diminished. Increased separation of fluid into the bowel and diminished absorption from the bowel are the factors regulating the fluid content. Nothing can be determined, however, by the examination of the amount of total solids, as regards the pathologic conditions accountable for an increase or a decrease.

Total Nitrogen.

The determination of the total nitrogen must always be made in estimating the metabolism in any given case. In so doing it is likewise essential that one should know the absolute nitrogen intake, as without such a factor nothing can be learned from the excretion either in the urine or in the feces. The daily excretion of nitrogen in a fasting condition varies from 1 to 4 grams, on a mixed diet this may run from 3 to 7 grams, while on a vegetable diet the nitrogen of the feces may be as high as 10 grams, a pure meat diet yielding between

2 and 6 grams. In pathologic conditions this amount is practically always increased, due both to lack of digestive power and to decrease in the absorptive function of the intestines. Outside of metabolic experiments the absolute amount of nitrogen of the feces has no great diagnostic importance beyond showing some perversion of intestinal activity.¹ In insufficiency of the pancreatic secretion, we are apt to find the greatest loss of nitrogen by way of the feces.

The constituents which go to make up this total nitrogen are the various undigested protein bodies, such as albumin, globulin, nucleoprotein, nuclealbumin, and gelatin, along with partially or completely digested products of protein origin, bacteria, and secretions from the intestines. These latter bodies embrace albumoses, peptone, the various amino-acids, and the hexone bases along with a certain amount of ammonium salts. The chemical properties of these substances, as well as the methods of their chemical detection and estimation, must be looked for in works on physiological chemistry. The bacteria form a large percentage of the total nitrogen.² It is rare, even in metabolic work, that the nitrogen partition of the feces is determined. Much more importance attaches, with our present knowledge, to such division of the nitrogenous material of the urine. Likewise the products of abnormal decomposition of protein material, taking place in the bowel with the formation of such products as indol, skatol, and phenol, are rarely searched for in the feces.³ Our clinical knowledge of the importance of these substances is confined largely to their detection and estimation in the urine. In the study of the metabolism of various cases of cystinuria, which is associated with abnormal protein disintegration, certain diamines, such as cadaverin and putrescin, have been isolated from the feces by Udransky and Baumann.⁴

Fat.

The chemical estimation of the amount of fat is of importance only in metabolic work, as in the more direct clinical examinations this substance is detected by macroscopic and microscopic methods. It is, however, essential that the loss of fat by way of the feces should be known before a proper metabolic balance can be struck.

The dried feces is treated with a small amount of 1 per cent. acid-alcohol and evaporated to dryness in order to convert any soaps which may be present into the fatty acids. The dry residue thus treated is then placed in a Soxhlet apparatus and extracted with ether for at least 72 hours. The ether, which has taken up the free fat and the fatty acids, is then evaporated and the residue of fats weighed. From this weight the percentage of fat may be deter-

¹ See McCrudden and Fales, *Jour. Exper. Med.*, 1912, XVII, 20 and 24; also Koplik and Crohn, *Am. Jour. Dis. Children*, 1913, V, 36; Gamble, *Ibid.*, 1915, IX, 519; Van Slyke, Courtney and Fales, *Ibid.*, 533.

² Mattill and Hawk (*Jour. Exper. Med.*, 1914, XIV, 433) shows that the bacterial nitrogen constitutes 53.9 per cent. of the total fecal nitrogen.

³ See Moewes, *Ztschr. f. exp. Path. und Ther.*, 1912, XI, 555. See, also, Rodella, *Arch. f. Verdauungskr.*, 1914, XX, 657; Whipple (*Jour. Am. Med. Assn.*, 1915, LXV, 476) believes that the intoxication of intestinal obstruction is due to the absorption of a primary proteose.

⁴ See Hopkins (*Arch. Int. Med.*, 1913, XI, 300) for a discussion of the presence of hemolysins in fecal extracts.

mined, and, knowing the original dry weight of the total 24-hour feces, the total fat lost by way of the feces may be readily calculated.¹

The amount of fat in the feces will depend much upon the amount and quality of the diet. In fasting conditions the amount is usually about 1 gram. On diets poor in fat the amount in the feces may exceed that of the diet, thus indicating a loss of fat from the body. It seems to be a general rule that the higher the melting point of the fat of the diet the greater will be the loss in the feces. Thus on a diet containing only butter as a fat about 4 per cent. of the intake will be lost, while with pork fat the loss may be as high as 13 per cent. It is impossible to say, *a priori*, just what the fat content of a normal feces would be. With the ordinary diet of our country it would range from 2 to 7 per cent. of the intake, which should be somewhere about 100 grams of fat per day, representing a loss of from 2 to 7 grams daily.

Carbohydrates.

As previously stated, carbohydrates may appear in the feces either in the form of starch or of cellulose. Only in exceptional conditions do we find any of the monosaccharids present. Disaccharids, such as lactose and maltose, may be occasionally found, but only when there is a combination of insufficiency, both of a salivary and pancreatic ferment. The presence of any appreciable amount of starch granules must be considered pathological, while the amount of cellulose will depend upon the amount in the food as well as upon the preparation of the food and its mastication.

Nothing is to be learned, from the clinical standpoint, by the determination of the absolute amount of carbohydrate in the feces. In the study of the utilization of food substances by the system and in general metabolic work it is, however, necessary to know just how much of the carbohydrate intake is absorbed. This may be determined indirectly by subtracting from the weight of the dry feces the sum of the protein, fat, and ash. This result will represent a much higher figure for carbohydrates than the one obtained by direct determination. In the direct determination much difference exists between the soluble forms, and the insoluble cellulose. In the latter case we have to do with a useless form of carbohydrate and should, therefore, direct our attention rather toward the estimation of the amount of undigested or partially digested starch. We should determine the amount of cellulose and subtract this factor from the total carbohydrate obtained in the previous subtraction. This corrected figure will represent more nearly the true amount of carbohydrate than will the former, although for scientific purposes a direct determination is essential.

The starch may be estimated by taking a weighed amount of the dry feces and treating with 50 c.c. of 10 per cent. HCl. This is then boiled for one-half hour in order to convert the starch and digested portions of starch into the monosaccharids. This acid solution is then filtered and washed

¹ See Cowie and Hubbard, *Am. Jour. Dis. Child.*, 1913, VI, 192; also, Bloor, *Jour. Biol. Chem.*, 1913, XV, 105; *Ibid.*, 1913, XVI, 517; Saxon, *Ibid.*, 1914, XVII, 99; Gephart and Csonka, *Ibid.*, 1914, XIX, 521; Smith, Miller and Hawk, *Jour. Biol. Chem.*, 1915, XXI, 395; Laws and Bloor, *Am. Jour. Dis. Child.*, 1916, XI, 229; Dyer, *Jour. Biol. Chem.*, 1917, XXVIII, 445; Holt, Courtney & Falls, *Am. Jour. Dis. Child.*, 1919, XVII, 38; Gardner, *Biochem. Jour.*, 1921, XV, 244.

with sufficient water to make the total approximately the same as that of the original solution. The filtrate is then neutralized with sodium hydrate and made up to exactly 100 c.c. in a volumetric flask. The sugar in this solution is then determined by the methods outlined under Urine. This method, although not absolutely accurate, will yield results sufficiently correct for ordinary purposes. More accurate results will be given by the Volhhard-Pflüger method to be discussed later. The amount of glucose, as determined by either of these methods, will give, if multiplied by 0.94, the amount of starch in the feces taken. The percentage may then be readily calculated and the total quantity of the 24-hour excretion of starch determined.

The fermentation method of Schmidt was advanced to permit of rough estimation of the presence of pathological amounts of starch, which, under ordinary circumstances, should have been digested. The principle of the method is the estimation of the amount of gas produced by the action of the intestinal bacteria upon the sugar which is formed from the starch by the intestinal amylolytic ferments. Prior to this determination the patient is placed upon the test diet outlined on page 106. The test is made as follows: Approximately 5 grams of the moist stool are placed in the vessel *a* which is then filled with water and the contents thoroughly mixed (see cut). The stopper is then placed in the fermentation flask in such a way that no air-bubbles are left. The tube *b* is filled with tap-water and closed with a small stopper without the inclusion of any air-bubbles. The tube *c* is now placed in position as shown in cut and the whole apparatus put in an incubator for 24 hours. The tube *c* has a small opening in the top so that water may be readily forced from the tube *b* into *c* by pressure of the gas produced. According to Schmidt 0.1 gram of starch will cause the tube *b* to show about one-half its volume of gas. Normally, a positive result is said to occur when the tube is one-fourth to one-third filled with gas. The material in the fermentation flask *a* should be tested with litmus-paper after the test is complete to obtain the reaction of the mixture. If the gas formation is, as it should be, due to carbohydrate fermentation there will be a slight increase in the acidity of the mixture, while if it be due to protein putrefaction the reaction will show a slightly increased alkalinity.

The various decomposition products of carbohydrate digestion and fermentation are rarely of importance in fecal examinations. These products consist of volatile fatty acids, lactic acid, saccharic acid, alcohol and aldehyde, and the conjugated glycuronic acids. Tests for these various substances will be found in various parts of this work or in works on physiological chemistry to which the reader is referred.¹

¹ See Fischer, *Ztschr. f. exper. Path. u. Therap.*, 1913, XIV, 179.

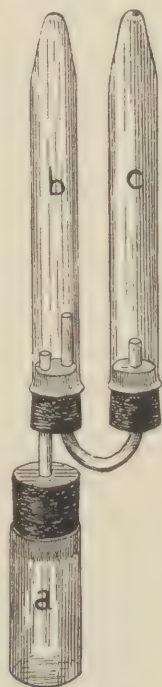


FIG 21.—
Schmidt's fermentation apparatus

From the clinical standpoint the gases produced by the processes of fermentation and putrefaction in the bowel have little value. The same is to be said of the quantitative estimation of the inorganic constituents of the feces. These latter may be determined by methods outlined in quantitative analysis.

The various ferments found in the intestinal canal may be detected in the feces, but such examinations are at present of little clinical value. Methods of isolating these ferments and of determining their activity belong more to physiological chemistry than to clinical diagnosis and will, therefore, be neglected.¹

Phenoltetrachlorphthalein Test.

There is no question but that the liver has a more varied and manifold functional activity than any other organ of the body. Slight disturbances in hepatic activity are a matter of relatively frequent occurrence, but these are not attended with any marked perversion of function as the larger portion of the parenchyma of the liver may not be involved. In disease, however, of the liver the character of the functional changes will vary with the nature of the underlying pathological condition. There is not so much difficulty in determining, from clinical and laboratory data, that hepatic disease exists as there is in estimating the intensity of the disease and the degree of disturbance of the functional activity. For this reason various attempts have been made to devise tests which would indicate such functional variations sharply and permit of a judgment concerning their intensity. While many of these tests have been proven of questionable value, some of them, such as the levulose and galactose tolerance test as well as the urobilinogen estimation, are of some importance in diagnosis and prognosis and will be discussed in the section on Urine. These tests have, for the most part, lacked a quantitative element, indicating more the presence than the extent of the disease.

Rowntree, Hurwitz and Bloomfield have introduced a test for the functional activity of the liver, which is probably the best at our disposal. They take advantage of the fact, discovered by Abel and Rowntree, that phenoltetrachlorphthalein, a drug first prepared by Orndoff and Black, escapes from the body only in the bile and may be recovered from the feces to a considerable extent. Under normal conditions the drug is not excreted to any appreciable extent in the urine. In other words, the liver exerts a specific action in excreting this dye, just as does the kidney in eliminating phenolsulphonephthalein. With this fact as a basis, these workers were able to show that a decreased capacity for its excretion was a resultant of lowered functional activity due to disease, and that minimal amounts of the dye escaped with the urine in pathological conditions of the liver. By direct estimation of the amount of this dye excreted by the bile and carried into the intestines one may readily estimate the presence and, to some extent, the degree of the functional disturbance of the liver.

The solution used for the injection is an aqueous solution of the disodium

¹ See Crohn, *Am. Jour. Med. Sci.*, 1913, CXLV, 393; also, Chace and Myers, *Arch. Int. Med.*, 1913, XII, 628; Brown, *Bull. Johns Hopkins Hosp.*, 1914, XXV, 200; Einhorn, *Am. Jour. Med. Sc.*, 1914, CXLVIII, 490; Crohn, *Ibid.*, 839; *Arch. Int. Med.*, 1915, XV, 581.

salt of phenoltetrachlorphthalein. This is prepared as follows: 2.5 grams of the phenoltetrachlorphthalein are placed in a 200 c.c. flask with 5 c.c. of an 8 per cent. (2N) sodium hydrate solution and 45 c.c. of freshly distilled water. Boil for 20 minutes under a reflux condenser. Filter the solution into a 100 c.c. flask, when it is ready for use. It is an intense purplish-red solution, which does not keep for many days owing to the precipitation of the dye by the CO_2 of the air. Hence relatively fresh solutions must be used.

Method of Administration.

Eight c.c. of the above solution, representing 400 mg. of phenoltetrachlorphthalein, are administered intravenously to the patient, using all the anti-septic and aseptic precautions which this method involves. The funnel and tubes are filled with sterile physiologic salt solution and, after the flow is established, the solution of the dye is added. Fifty to 100 c.c. of salt solution are used, the dye being washed in with the salt solution until the fluid entering the vein is colorless.

Collection of Material.

Before beginning the administration of the dye, active purgation of the patient should be induced and should be continued throughout the time of observation. If little feces can be obtained, enemata should be employed. Collect all the fecal material which passes for 48 hours and the urine for 24 hours.

Place the total 48-hour specimen of feces in a large bottle and dilute it with 1 to 1.5 liters of water, depending on the amount of feces. Place this mixture in a shaking machine for 5 to 20 minutes. Without allowing time for sedimentation, one-tenth of the total mixture is placed in a liter flask and to this is added 5 c.c. of 40 per cent. sodium hydrate, which causes the mixture to take on a dirty red color. Now dilute the mixture to 1 liter with water, stopper the flask, and shake thoroughly. One hundred c.c. of this preparation are placed in a 200 c.c. flask and 5 c.c. of a saturated solution of basic lead acetate are added. Now add 5 c.c. of 40 per cent. sodium hydrate solution to elicit the maximum intensity of red color. If the amount of NaOH added does not seem sufficient, add a little more, being careful not to redissolve the precipitate present.¹ Make up the contents of the flask to 200 c.c., shake and filter a small portion for comparison with a standard solution of phenoltetrachlorphthalein. This comparison is made in a colorimeter, either of the Duboscq type or of the type of Rowntree and Geraghty's modification of the colorimeter of Autenrieth-Königsberger. For comparison one may use 0.4 c.c. of the original solution, making this up to a liter and adding enough NaOH to insure the maximum color.

There are certain elements connected with this test that are not very desirable. It involves intravenous injection, which is followed in many cases by thrombosis at the site of injection. This is not sufficient to occasion serious discomfort, but the fact should be remembered and its possibilities

¹ If difficulty is experienced on account of the quality of the color at this point, add to the 100 c.c. of mixture 5 to 10 c.c. or more of a calcium chlorid mixture (CaCl_2 , 90 grams; conc. NH_4OH , 10 c.c.; water, 50 c.c.) until the best color appears. Dilute to 200 c.c., allow the mixture to stand from 1 to 24 hours and decant the supernatant fluid for comparison.

taken into consideration. Further, it necessitates a careful collection of feces for 48 hours. The quality of the red color obtained in the fecal extract in some cases is such that accurate quantitative determinations are difficult.

The results with this test show that a phenoltetrachlorophthalein output in the feces below 30 per cent. or its appearance in the urine is quite infrequent in health. The output of a less amount is relatively frequent in hepatic disease, although a normal output of 30 per cent. does not exclude liver lesions. The output of the dye in the feces is independent of the quantity of bile excreted, although it is evident that none of the dye will reach the intestines in cases of obstruction of the duct. Functional changes, as evidenced by a lessened output of the dye in the feces, have been most marked in cirrhosis, carcinoma and in cachexias associated with severe anemias. Chronic passive congestion does not give rise to a much changed excretion, although marked hepatic congestion associated with insufficiency of the myocardium is usually accompanied by a lowered output. Although this test is not to be regarded as diagnostic in all cases of perversion of hepatic activity, yet it offers much evidence which can be obtained by very few other tests so far advanced.¹

V. BACTERIOLOGY OF THE FECES

The bacteria of the intestinal canal are of many types, many of which are purely saprophytic, while others may or may not be pathogenic.² The number of these bacteria is usually enormous. One may determine the amount of these bacteria by the method of Strasburger, who uses the following technic: Two c.c. of feces are rubbed up in a porcelain mortar with 30 c.c. of $\frac{1}{2}$ per cent. hydrochloric acid. This mixture is then placed in centrifugal tubes and whirled for one minute. The bacteria will remain in suspension in the liquid which is poured off from the sediment. The sediment is again rubbed up with a little hydrochloric acid and again centrifuged, the liquid being added to the first portion. This procedure may be repeated until the fluid no longer becomes turbid on centrifuging. This acid solution, holding in suspension the bacteria, is then mixed with an equal portion of ordinary alcohol and placed in a beaker which is allowed to remain on a constant water-bath at 40° for 24 hours. At the end of this period a portion of the fluid may be evaporated and more alcohol added. This mixture is then placed in the centrifuge tubes and whirled for several minutes. The bacteria are now deposited and the supernatant fluid is poured off and mixed so that it may be again centrifuged. The bacterial sediment is washed with alcohol and ether in the centrifuge tube and is then placed in a weighed dish. This is

¹ Orndoff and Black, *Jour. Am. Chem. Soc.*, 1901, XLI, 349; Abel and Rowntree, *Jour. Pharmacol. and Exper. Therap.*, 1909, I, 233; Whipple, Mason and Peigntal, *Bull. John. Hopkins Hosp.*, 1913, XXIV, 207; Rowntree, Hurwitz and Bloomfield, *Ibid.*, 327; Whipples Peigntal and Clark, *Ibid.*, 343; Whipple and Christman, *Jour. Exper. Med.*, 1914, XX, 297; Chesney, Marshall and Rowntree, *Jour. Am. Med. Assn.*, 1914, LXIII, 1533; Sisson, *Arch. Int. Med.*, 1914, XIV, 804; Whipple and Speed, *Jour. Exper. Med.*, 1915, XXI, 203; McLester and Frazier, *Jour. Am. Med. Assn.*, 1915, LXV, 383; Kahn and Johnston, *New York Med. Jour.*, 1915, CII, 848; McNeil, *Jour. Lab. and Clin. Med.*, 1916, I, 822; Foster and Kahn, *Ibid.*, II, 25; Kahn, *Jour. Am. Med. Assoc.*, 1921, LXXVII, 41; Aaron, Beek and Schneider, *Ibid.*, 1631.

² See MacNeal and Chace, *Arch. Int. Med.*, 1913, XII, 178; also, MacNeal, *Am. Jour. Med. Sc.*, 1913, CXLV, 801; Rahe, *Jour. Infect. Dis.*, 1915, XVI, 210.

dried at 100° and again weighed. In order to determine the amount of bacteria as compared with the total feces, the dry weight of 2 grams of the fresh feces is determined as previously outlined. Knowing these factors, the percentage of bacteria in the dry feces may be easily calculated. Strasburger found that this was approximately one-third the weight of the dry stool and represented about 8 grams per day.¹ As this dry feces is used for the determination of total nitrogen, we must bear in mind that the bacteria of the feces will represent about one-half of the total nitrogen of the feces.

It is to be said that the bacterial flora of the intestine is so varied that very careful and long-continued work is necessary to isolate a special organism. From the standpoint of preventive medicine it should always be remembered that the feces of typhoid or cholera patients contain large numbers of

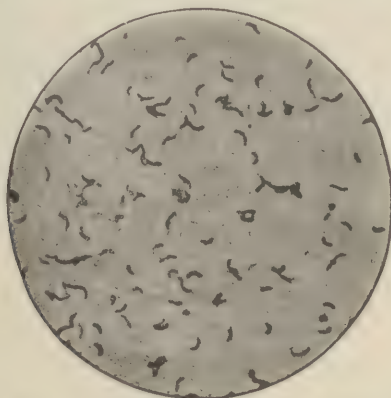


FIG. 22.—Cholera spirilla. (Pitfield.)

typhoid bacilli and cholera spirilla, so that measures should be taken to treat properly such ejecta as soon as voided.² While the majority of the bacteria of the intestines are saprophytic, being introduced with the food or drinking-water, yet we find the *Bacillus coli communis* as a normal habitant of the intestine. This organism is usually harmless, but may, under certain conditions, become distinctly pathogenic, many obscure cases of gall-bladder infection, for instance, being traceable to this organism.

The presence of non-pathogenic organisms seems to be an essential for proper performance of intestinal function. This idea, originally advanced by Pasteur, has been denied by Schottelius, Nuttall, and Thierfelder, although their experiments extended only over periods of 17 days. These intestinal bacteria not only aid digestion, but also prevent a certain amount of abnormal decomposition, owing to the fact that they inhibit the development of foreign types to a large extent, especially under normal conditions. These normal bacteria may under certain conditions, however, become pathogenic, but only when the intestinal wall loses its continuity. In case the normal bacteria of

¹ See Mattill and Hawk, *Jour. Exper. Med.*, 1911, XIV, 433.

² Moody and Irons (*Jour. Inf. Dis.*, 1920, XXVII, 363) have shown the presence in the feces of hemolytic streptococci in scarlet fever. Several other types of streptococci, as well as staphylococci, are likewise found in the feces. See Oppenheim, *Ibid.*, 1920, XXVI, 117; Davis, *Ibid.*, 171.

the intestine become too numerous, the products formed by their activity upon protein material may be absorbed and bring about certain toxic effects.¹ It is interesting in this connection to find that the bacteria are diminished in amount in chronic constipation, according to Strasburger. The toxic symptoms must, therefore, be referable to the absorption of other than bacterial products of decomposition, or the products have increased toxicity. For the best conditions to exist the symbiotic relations of the intestinal bacteria should be such that there are neither too few nor too many bacteria.

The normal adult stool shows a preponderance of Gram-negative organisms (colon bacilli). Pathologically an increase in this type may be noted (cholera spirilla) or Gram-positive organisms may prevail. This Gram-staining variation in the bacteria of the feces is, also, largely influenced by changes in diet, bacteria of the *Bacillus acidophilus* and *bifidus* types becoming quite prominent under a substitution of a low protein for a high protein diet.² A proteolytic or putrefactive flora results from high protein feeding and a carbohydrolytic or fermentative active flora develops when the protein intake is low and the diet consists largely of carbohydrates. It has been shown by Rettger and Cheplin that the incorporation of lactose and dextrin into the diet leads to a marked development of the *Bacillus acidophilus* and to the repression of the proteolytic intestinal bacteria.

(a) The Cholera Spirillum.

This organism, known as the comma bacillus, is about 2 microns long and $\frac{1}{2}$ micron thick. It is very actively motile and has a single delicate flagellum at one end. It stains easily with the ordinary bacterial stains and is decolorized by Gram's method. The cultural peculiarities of this spirillum will be discussed in the last section of this book. This organism is usually recognizable in the stools of Asiatic cholera, which are the characteristic "rice-water" stools. The blood serum of patients affected with cholera will show very characteristic agglutination of cultures of these organisms. The blood is usually used in a dilution of 1 to 15, the reaction being observed in from 5 to 20 minutes.

Closely related to this comma bacillus of Koch is the bacillus of Finkelf-Prior. This latter organism may be distinguished from the spirillum of Asiatic cholera by its morphology, the organism being larger and thicker than

¹ See Zuntz, *Die Naturwissenschaften*, 1913, I, 7.

² See Herter and Kendall, *Jour. Biol. Chem.*, 1910, VII, 203; Rettger and Horton, *Centralbl. f. Bakteriolog., Orig.*, 1914, LXXIII, 362; Osborne and Mendel, *Jour. Biol. Chem.*, 1914, XVIII, 177; Logan, *Jour. Path. and Bacteriol.*, 1914, XVIII, 527; Blatherwick and Hawk, *Jour. Am. Chem. Soc.*, 1914, XXXVI, 147; Rettger, *Jour. Exper. Med.*, 1915, XXI, 365; Torrey, *Jour. Infect. Dis.*, 1915, XVI, 72; Logan, *Lancet*, 1916, II, 824; Nissle, *Deutsch. med. Wchnschr.*, 1916, XLII, 1181; Hull and Rettger, *Jour. Bacteriol.*, 1917, II, 47; Sisson, *Am. Jour. Dis. Child.*, 1917, XIII, 117; Porter, Morris and Meyer, *Ibid.*, 1919, XVIII, 254; Morris, Porter and Meyer, *Jour. Infect. Dis.*, 1919, XXV, 349; Torrey, *Jour. med. Res.*, 1919, XXXIX, 415; Mendel, *Am. J. Med. Sc.*, 1919, CLVIII, 297; Distaso and Sugden, *Biochem. Jour.*, 1919, XIII, 153; Rettger and Cheplin, *A Treatise on the Transformation of the Intestinal Flora, with special Reference to the Implantation of Bacillus Acidophilus*, Yale University Press, 1921; Gompertz and Vorhaus, *Med. Record*, 1921, C, 497; Davison and Rosenthal, *Am. Jour. Dis. Child.*, 1921, XXII, 284; Hoefert, *Ztschr. f. klin. Med.*, 1921, XCII, 221; Adam, *Ztschr. f. Kinderhke.*, 1921, XXIX, 50, 65 and 306; *Ibid.*, XXX, 265; *Ibid.*, 1922, XXXI, 331; Cannon, *Jour. Inf. Dis.*, 1921, XXIX, 369; Rettger and Cheplin, *Arch. Int. Med.*, 1922, XXIX, 357.

the comma bacillus, and by the appearance of the stab cultures on gelatin. The cholera spirillum forms a typical funnel-shaped depression, while the bacillus of Finkler-Prior shows a stocking-like depression. The Finkler-Prior bacillus is found in cases of cholera nostras. It may be necessary for the absolute identification of these organisms to apply bacteriolytic tests with serum of animals immunized against a specific type of organism.¹ (See Chapter XI.)

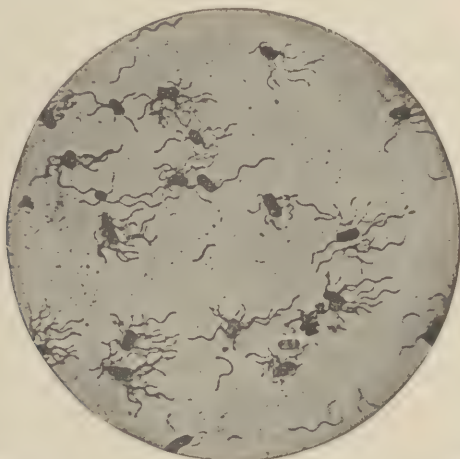


FIG. 23.—*Bacillus typhosus*, stained to show flagella.
(Oertel after Fränkel and Pfeiffer.)

(b) Typhoid Bacillus.

This organism, discovered by Eberth, is so similar in morphology to numerous other organisms, especially to the *Bacillus coli communis*, that simple staining methods do not suffice for its detection. The characteristic stool of typhoid fever is a copious watery stool, having a strong odor and an alkaline reaction. This stool is known as the "pea-soup" stool, and may be tinged with blood and contain many pus-cells.

The typhoid bacilli are medium-sized organisms with rounded ends, generally short, but sometimes long or thread-like and frequently showing faintly stained, sharply defined areas in their protoplasm. They are actively motile and have both polar and lateral flagella. This organism stains with the ordinary dyes and is decolorized by Gram's method.

Method of Drigalski and Conradi.²

Three pounds of minced beef are mixed with 2 liters of water and allowed to stand overnight. The beef is then pressed and the juice boiled for one hour and filtered. To the filtrate are added 20 grams of Witte's peptone, 20 grams of nutrose, and 10 grams of sodium chlorid. Boil this mixture one hour and filter. To the filtrate 60 grams of agar are added and the mixture boiled for three hours, one of which should be in the autoclave. Slightly

¹ See Public Health Reports, 1912, XXVII, 371.

² Ztschr. f. Hyg. u. Infektionskrankh., 1902, XXXIX, 283. For a discussion of the use of benzin in differentiating members of this group see Benians, Ztschr. f. Chemotherap., 1913, II, 28; Bierast, Centralbl. f. Bakteriöl, I. Abt., Orig., 1914, LXXIV, 348; Schmitz, Münch. med. Wchnschr., 1914, LXI, 2115; Jaffé, Wien. klin. Wchnschr., 1915, XXVIII, 418. Hall, Berl. klin. Wchnschr., 1915, LII, 1326.

alkalinize the mixture to litmus-paper, filter, and boil for one-half hour. To this hot agar solution, which should be now about 60°, add the following litmus-lactose solution. Two hundred and sixty c.c. of litmus solution are boiled for 10 minutes, after which 30 grams of chemically pure lactose are added and the mixture boiled for 15 minutes longer. This litmus-lactose solution is added while boiling to the hot agar solution, the mixture being well shaken and again faintly alkalinized to litmus. Four c.c. of a hot sterile 10 per cent. solution of sodium carbonate and 20 c.c. of a freshly prepared 0.1 per cent. solution of crystal-violet B. (Höchst) in warm sterile distilled water are then mixed in. This medium may be poured directly into plates or kept in flasks. It soon hardens to a firm mass and does not become dry readily.

The principle upon which the use of this medium depends is that in the presence of both lactose and protein the colon bacillus will first attack the milk-sugar, while the typhoid bacillus will act upon the protein. In the presence of litmus the colonies of colon bacilli become distinctly red, while those of the typhoid bacillus are blue. The crystal-violet inhibits the growth of many of the other organisms, especially of the acid-producing type.¹

If the stool be fluid, as is usually the case, one series of two plates is inoculated with the undiluted stool, another with a stool diluted with 10 volumes of sterile normal salt solution, while other dilutions, such as 1 to 100 and 1 to 1000 may also be made. If the stool is solid it is rubbed up into a homogeneous mass with sterile salt solution and the various dilutions made as above. In making the inoculations from the stools, the material is rubbed over the surface of the medium, the plates being left open to allow the surface to dry. The dry plates are then placed in the incubator at 37° and examined at the end of 24 hours. Any contamination of the media will be killed by the crystal-violet.

It frequently happens that certain strains of the paratyphoid bacillus develop blue colonies in this medium. It is, therefore, necessary for absolute differentiation that the agglutination test described under blood be carried out. In this way the typhoid bacillus may be absolutely identified, especially in the presence of its well-known morphological characteristics.²

¹ Quite an extensive amount of research has centered about the use of brilliant green in culture media used for isolation of typhoid bacilli. This dye seems to afford an excellent enrichment medium. See Krumwiede, Pratt, and McWilliams, *Jour. Infect. Dis.*, 1916, XVIII, 1; Teague and Clurman, *Ibid.*, 647 and 653; Robinson and Rettger, *Jour. Med. Res.*, 1916, XXXIV, 363; Teague and Clurman, *Ibid.*, 1916, XXXV, 107; Meyer and Stickel, *Jour. Infect. Dis.*, 1918, XXIII, 48; Krumwiede, Kohn, Kuttner, and Schumm, *Ibid.*, 275. For other methods in connection with the isolation of this organism see Kemper, *Jour. Infect. Dis.*, 1916, XVIII, 209; Tonney, Caldwell, and Griffen, *Ibid.*, 239; Holt, Harris, and Teague, *Ibid.*, 596; Ecker, *Ibid.*, 1918, XXII, 95; Hulton and Frankel, *Ibid.*, 1918, XXIII, 380; Kligler, *Jour. Exper. Med.*, 1918, XXVIII, 319; Pacini and Russell, *Jour. Biol. Chem.*, 1918, XXIV, 43; Bolten (Reprint 453 from Public Health Rep., U. S. P. H. Service, 1918) finds that the typhoid organism resists freezing for some time and points out that infected ice cream would be a very effective agent for distributing infection. See, further, Morishima, *Jour. Bact.*, 1921, VI, 275; Beckwith and Lyon, *Jour. Inf. Dis.*, 1921, XXVIII, 62; Schoenholz and Meyer, *Ibid.*, 384 and 588; Chesney, *Jour. Exp. Med.*, 1922, XXXV, 181.

² For a discussion of the differentiation and characteristics of Paratyphoid bacillus A and B, see *Bacteriology of Blood*. Ravenel (*Jour. Am. Med. Assoc.*, 1921, LXXVI, 720) reports an interesting case in which the typical lesions of typhoid fever were produced by the *Bacillus faecalis-alkaligenes*.

Method of Kendall and Day.

This method¹ permits of rapid diagnosis and is especially of importance in the examination of stools of suspected "carriers." The culture medium is a modification of that of Endo. Plain nutrient sugar-free agar is prepared as follows: 1000 c.c. of cold tap water, 15 grams of powdered agar, 10 grams of Witte's peptone, and 3 grams of Liebig's extract of beef are mixed and cooked in the double boiler for one hour. Just alkalize to litmus by careful addition of sodium hydrate solution, cook for 15 minutes and filter through absorbent cotton. This filtrate is then stored in flasks containing known amounts (100 c.c.) and is sterilized in the autoclave. When it is desired to use this media, add 1 gram of chemically pure lactose to each 100 c.c. and heat in the sterilizer until the media is melted and the lactose thoroughly distributed. Now prepare a mixture of 1 c.c. of a filtered 10 per cent. alcoholic solution of fuchsin and 10 c.c. of a fresh 10 per cent. aqueous solution of sodium sulphite.² Add 1 c.c. of this sterilized and decolorized fuchsin mixture to each 100 c.c. of the liquid lactose media and mix thoroughly. Plates are poured and allowed to harden (with the covers removed) in the incubator for 30 minutes, when they are ready for inoculation.

A small portion of feces is emulsified in 10 c.c. of sugar-free³ bouillon and incubated for 1 hour at 37°C. This suspension is rubbed gently but firmly over the surface of the agar plates by means of sterile hooked glass rods and incubated for 18 hours at 37°C. The translucent, colorless, "dew-drop" colonies are removed to small tubes, which contain 1 c.c. of sugar-free bouillon and have been kept at 37°C. prior to use.⁴ Incubate for 2 hours and make agglutination tests, using a known serum of high agglutinating power (see blood).

(c) The Bacillus of Dysentery.

Bacillary dysentery is distinctly different from the amebic type of dysentery. The bacillus dysenteriae, or Shiga's bacillus, is now generally recognized as the specific organism of this type of dysentery.

Flexner found a similar bacillus in the dysentery of the Philippines, while Kruse has found practically the same type in Germany. In the United States Flexner and Harris find an organism which answers the description of the ordinary Shiga bacillus. The variation between the different organisms described is very slight, so that one may generally consider them as varieties of the same species, although certain cultural and agglutinating differences are observed.

¹ Jour. Med. Research, 1911, XXV, 95. See, also, Schmidt, Deutsch. med. Wchnschr., 1915, XLI, 33; Hirschbruck and Diehl, *Ibid.*, 606.

² If preferred Andradi's indicator (a 0.5 per cent. aqueous solution of acid fuchsin decolorized by the careful addition of 10 per cent. sodium hydrate solution) may be substituted. See Kahn, Jour. Bacteriol., 1918, III, 547.

³ This sugar-free broth is specially prepared to remove the muscle-sugar. Meat extract is inoculated with colon bacilli and incubated for 16 hours. Boil and strain through cloth. The filtrate is then made up as ordinary bouillon.

⁴ Or the colonies may be transferred to Russell's medium (litmus-lactose-glucose agar) as recommended by Lumsden (Pub. Health Reports, 1912, XXVII, 789). See also Kendall and Ryan, Jour. Infect. Dis., 1919, XXIV, 400, for a double sugar medium of saccharose (1 per cent.)—mannitol (0.1 per cent.) agar; Nichols, Jour. Inf. Dis., 1921, XXIX, 82.

The Shiga bacillus is a short rod with rounded ends, much resembling the typhoid bacillus but much plumper. It does not seem to have very active motility as far as progression is concerned, although it does show a high degree of molecular motility. It stains with the usual dyes and is decolorized by Gram's method. The only sure method of identification of the various types of the dysentery bacillus seems to be the agglutination test.

The fecal material is best obtained by curettage of the rectum, as these bacilli seem to be present in the mucus and are thus more easily concentrated. The cultural peculiarities of this organism will be found in a later section.

(d) **The Tubercle Bacillus.**

The examination of the stools for tubercle bacilli is not always satisfactory. The enormous number of bacteria of the feces may prevent a recognition of the tubercle bacillus even though it is present. In the feces we find certain organisms which are acid-fast, such as the timothy bacillus, which is very closely related in morphology and staining characteristics to the tubercle bacillus. We should be on our guard, therefore, lest we make a wrong diagnosis.

If these organisms are present on repeated examination and there are clinical symptoms pointing to such a trouble of the bowel, one may give a presumptive diagnosis of tuberculosis, remembering that the tubercle bacilli may have come from swallowed tubercular sputum.¹

In selecting the material for examination, pick out the particles of mucus, especially those which are blood-stained or purulent. One may be much more certain of definite results if the antiformin or Petroff method be used upon the feces, which has been rubbed up with distilled water (see p. 18). The staining methods are as usual.

The *Bacillus aerogenes capsulatus* (*Bacterium Welchii*; gas bacillus) is fast coming into prominence as an etiologic factor in many cases of intestinal disturbance associated with diarrhea, although it is possibly a normal habitant of the intestinal tract of children.²

VI. PARASITOLOGY OF THE FECES

In the examination of the feces for parasites one should obtain the feces as fresh as possible. This is especially the case where the examination for protozoa such as the ameba is to be made. The feces should be kept in a warm vessel prior to the examination, as the motility of these unicellular organisms is shown only with great difficulty after they have become cold. The other types of protozoa are less sensitive to changes in temperature and show their active motility, providing the feces be examined soon after voiding.

¹ See Laird, Kite and Stewart, *Jour. Med. Research*, 1913, XXIX, 31; Bergstrand, *Hygiea*, 1915, LXXVII, 97; Petroff, *Jour. Exper. Med.*, 1915, XXI, 38; Keller and Moravek, *Med. Record*, 1915, LXXXVIII, 864; Lefller, *Hygiea*, 1916, LXXVIII, 1809; Brown, Sampson and Heise, *Am. Rev. Tuberc.*, 1920, IV, 451; Marchisotti, *Semana Med.*, 1921, XXVIII, 37; Carnot and Libert, *Bull. de la Soc. Med. des Hop. de Paris*, 1921, XLV, 1101.

² See Kendall and Smith, *Boston Med. and Surg. Jour.*, 1911, CLXIV, 306; Kendall, *Ibid.*, 1912, CLXV, 75; Orton, *Jour. Med. Research*, 1913, XXIX, 287; Knox and Ford, *Bull. Johns Hopkins Hosp.*, 1915, XXVI, 27; Morse, *Am. Jour. Med. Sc.*, 1915, CXLIX, 17.

It seems to be generally accepted that these organisms are the more easily found the more fluid, more mucoid, and more alkaline the feces. The particles for examination should be preferably the masses of mucus which can be found by careful search in the liquid stool. The organisms are best examined in their fresh condition, as the staining agents usually require fixation of the specimen and consequent death of the parasite.¹

In the examination of the feces for parasitic ova the following method of Yaoita (*Deutsch. med. Wchnschr.*, 1912, XXXVIII, 1540) is, in my opinion, the best. From several different parts of the fecal mass take portions the size of a pea. Place in a test-tube and add 5 c.c. of 25 per cent. antiformin solution. Mix thoroughly and warm but do not boil. Allow to cool, add 5 c.c. of ether and shake well. Filter the mixture through one layer of gauze and centrifuge the filtrate for one minute. Four layers are formed, in the lower of which are the ova along with the resistant cellulose, connective tissue and muscle fibers and salts. Pour off the supernatant fluid and treat the sediment with dilute HCl and a little ether. Shake well and centrifuge again. This latter process is repeated two or three times. The ova are thus freed from most of the other fecal material and may be detected readily by microscopic examination of the sediment.²

Kofoid and Barber, as a result of their extensive experience in examination of the stools of our troops during and since the war, have introduced what they style the "brine flotation-loop method" of examination for parasitic ova. The procedure is as follows: Mix a large fecal sample thoroughly in concentrated brine (saturated solution of NaCl) in a paraffined paper can of from 2 to 3 ounces capacity, forcing the coarse float below the surface by means of a disk of No. 0 steel wool, and then allow the can to stand one hour for the ova to ascend. The surface film is then looped off with wire loops, one half inch in diameter and examined on a slide without a cover glass. This method permits the rise of the ova to the surface of the brine mixture of feces, while the heavier material settles. It is simple and reliable for most ova, but its range of application seems to be limited by the type of ova. Kofoid and Barber themselves state that it is ineffective in the detection of ova of *Strongyloides* (which as a matter of fact rarely if ever appear in stools) while McDonald shows that the method does not detect infections with *Clonorchis* and *Fasciola* and that its effectiveness in detection of any operculate ova is doubtful.³

¹ Ross (Further Researches into Induced Cell-reproduction and Cancer, Philadelphia, 1911 and 1912) has advocated the method of embedding the suspected feces in agar-jelly which may be impregnated with the stain. See, also, Smithies, *Arch. Int. Med.*, 1912, IX, 736.

² See Fauntleroy and Hayden, *U. S. Naval Med. Bull.*, 1915, IX, 81; Hall, *Jour. Lab. and Clin. Med.*, 1917, II, 347; Bak, *Nederland, Tjdschr. voor Geneesk.*, 1917, II, 1117. Infection with intestinal parasites appears to be increasing. In this connection see Schloessmann, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1921, XXXIV, 1; Moore, *Jour. Am. Med. Assoc.*, 1922, LXXVIII, 1197.

³ Kofoid and Barber, *Jour. Am. Med. Assoc.*, 1918, LXXI, 1557; Kofoid and White, *Ibid.*, 1919, LXXII, 567; Kofoid et al., *Ibid.*, 1921, LXXIII, 1721; McDonald, *Jour. Lab. and Clin. Med.*, 1920, V, 386.

(1) Protozoa.

The protozoa are unicellular animal organisms. These, although living occasionally symbiotically, are more usually found as isolated single organisms. A few of these organisms are sufficiently large to be detected by the naked eye, but the majority are minute and require the finer microscopic detection. They consist essentially of a mass of protoplasm (cytoplasm or sarcode), with differentiation for functional purposes (organelles) of a variable character, constancy, and prominence. In the ameba, for instance, the sarcode may be separable into an internal distinctly granular portion known as the endosarc, and a peripheral clearer portion known as the ectosarc; a cell-membrane in some instances is a well-marked feature, while in others it is absent; and in some of the free-living protozoa special external coverings of chitinous, siliceous, or chalky composition enclose the protozoon. Of the various parts the nucleus is, after the cytoplasm, the most constant, varying much in appearance, shape, size and number in the individual form (single nucleus of variable size and shape; double or dimorphic nucleus, a macronucleus of vegetal character, a micronucleus with creative function; polymorphous nucleus, multiple nuclear granules more or less widely distributed in the cytoplasm).

Not uncommon examples of specialization are met in the contractile vacuoles, in pigment spots, in mouth-like ingestion foci and their pits on the surface of many forms with relatively firm cell membrane, in the anus-like excretory points of the same form, or the peripheral motor organelles, in the sucking tubes of the suctoria, and in the hook-like fixation apparatus of the gregarines.

Motile protozoa move in a variety of ways. The naked rhizopods move by a peculiar rolling due to currents in the internal substance of the cell or by the protrusion of the cell-substance as extensions or pseudopoda, these movements being always accompanied by change in the cellular shape of the animal. Ciliates and flagellates move through the activity of the special cuticular appendages known as cilia and flagella.

(a) Rhizopoda (Sarcodina).

Amœbina.

The classification of Amebæ has been a more or less difficult task, owing to lack of knowledge of the complete life cycle of many of the so-called species; the simple morphology of these organisms; the difficulties inherent in the study of such delicate cells; and the conflicting opinions of protozoologists as to the data upon which generic and specific classification should be based (Craig). The classification of Calkins,¹ although not entirely satisfactory appears to be the most logical. He divides the genus *Ameba* into seven genera as follows: *Ameba*, *Vahlkampfia*, *Nagleria*, *Craigia*, *Trimastigameba*, *Endameba* and *Parameba*. Only three of these, namely, *Vahlkampfia*, *Craigia* and *Endameba*, are of interest as parasitic amebæ of man. The only species of the first of these three latter genera to be actually identified as

¹ Tr. 15th. Internat. Cong. Hyg. and Demog., 1912, II, 287; See, also, Craig, Jour. Med. Research, 1917, XXXV, 425.

parasitic in man is *Vahlkampffia lobospinosa*, first described by Musgrave and Clegg¹ and later named by Craig. Of the second genus, two species have been shown to be parasitic, namely, *Craigia hominis* (*paramoeba hominis*) and *Craigia migrans*.² Of the genus *Endameba*, no less than twenty-six distinct species have been described as parasitic in man, but many of these have been determined to be identical with some of those previously described. In this number, only three of the species of endamebæ are of great interest to us, namely, *Endameba histolytica*, *Endameba coli*, and *Endameba gingivalis*. A further type, which is non-pathogenic, has been described by Weyon and O'Connor under³ the name *Endameba nana* and seems to be a very common intestinal ameba among the troops.

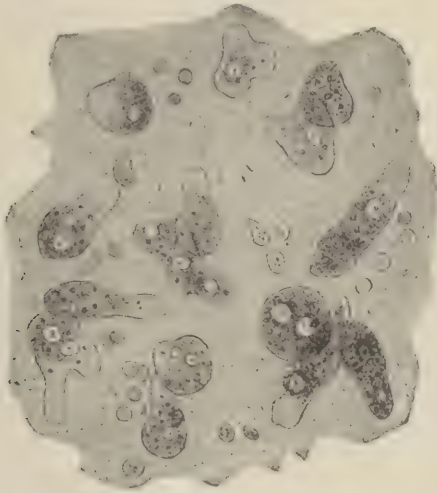


FIG. 24.—*Amœba coli*. (Hemmeter.)

(α) *Amœba coli* (*endameba histolytica*).

The ameba was first discovered in the large intestine by Lambl, although we are indebted to Lösch for the first accurate description of this organism. He did not believe it to be the cause of dysentery, but regarded it as a secondary invader. Since that time much work has been done, a controversy having arisen regarding its specificity. In a great many cases of dysentery this organism is not found, but in its stead bacteria, especially the Shiga bacillus, are present in large numbers. This bacillary dysentery is something entirely different from the amebic type of this disease. It is true that in certain cases of dysentery as well as in normal individuals, amebæ are found which are differentiated with great difficulty from the true *amœba coli*. There seem to be two distinct types: one pathogenic, to which Councilman and Lafleur give

¹ Bur. Gov't. Lab. Biol., Manila, 1904, No. 18; see, also, Craig, Arch. Int., Med., 1914, XIII, 737.

² Craig, Am. Jour. Med. Sc., 1906, CXXXII, 214; Barlow, Am. Jour. Trop. Dis. and Prevent. Med., 1915, II, 680.

³ Jour. Royal Army Med. Corps, 1917, XXVIII, 1; Kofoid, Kornhauser, and Plate, Jour. A. M. A., 1919, LXXII, 1721; Kofoid, Kornhauser and Swezy, Arch. Int. Med., 1919, XXVI, 35.

the name *amœba dysenteriae*, which Löscher styles *amœba coli*, and which Schaudinn designates *endamoeba histolytica*; a second type, which is non-pathogenic, has been styled by the first writers *amœba coli* Löscher and by Schaudinn *endamoeba coli*. Viereck¹ has described a form, which is pathogenic and is known as the *endamoeba tetragena*. This form is identical with the *endamoeba histolytica*. These forms have not been cultivated. The cultivated forms are non-pathogenic to man.²

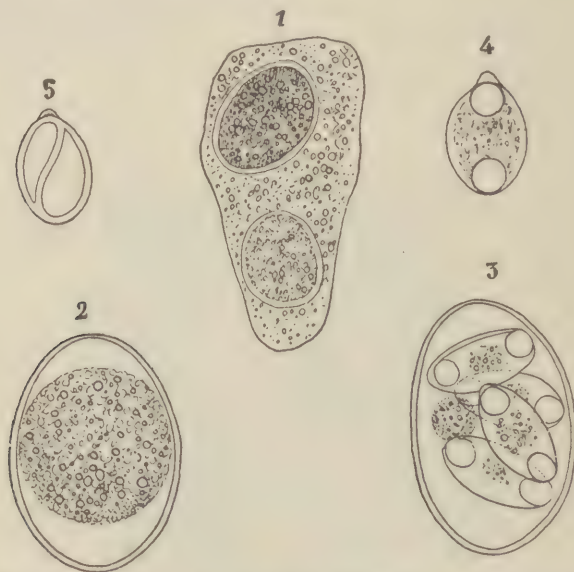


FIG. 25.—*Coccidium hominis*, from intestine of rabbit: 1, A degenerate epithelial cell containing two coccidia; 2, free coccidium from intestinal contents; 3, coccidium with four spores and residual substance; 4, an isolated spore; 5, spore showing the two falciform bodies— $\times 1140$. (Tyson after Railliet.)

One may sum up the points relative to the pathogenicity of the *endamoeba histolytica* as follows: (1) It appears in a form of dysentery which is anatomically characterized by peculiar ulcerations which are markedly different from the diphtheritic inflammatory processes of the bacillary dysentery. (2)

¹ Beiheft z. Arch. f. Schiffs. -u. Tropenhyg., 1907, I, 1.

² See Craig, Arch. Int. Med., 1911, VII, 362; Am. Jour. Med. Sc., 1912, CXLV, 83; Jour. Med. Research, 1912, XXVI, 1; War Dept., Office of Surgeon General, Bull. 2, 1913, 95; Jour. Am. Med. Assn., 1913, LX, 1353; South. Med. Jour., 1913, VI, 370; Jour. Infect. Dis., 1913, XIII, 30; Whitmore, Arch. Int. Med., 1912, IX, 515; Darling, Arch. Int. Med., 1913, XI, 1 and 495; Jour. Am. Med. Assn., 1913, LX, 1220; James, New York Med. Jour., 1913, XCVIII, 702; Couret and Walker, Jour. Exper. Med., 1913, XVIII, 252; Williams and Calkins, Jour. Med. Research, 1913, XXIX, 43; Giffin, Jour. Am. Med. Assn., 1913, LXI, 675; Walker and Sellards, Editorial, Jour. Am. Med. Assn., 1914, 300; James, Ann. Trop. Med. and Parasitol., 1914, VIII, 133; Craig, Arch. Int. Med., 1914, XIII, 737 and 917; Sellards and Baetjer, Am. Jour. Trop. Dis. and Prev. Med., 1914, II, 231; Bull. Johns Hopkins Hosp., 1915, XXVI, 45; Couret, Am. Jour. Trop. Dis. and Prev. Med., 1915, II, 450; Cutler, Jour. Path. and Bacteriol., 1918, XXII, 22; Parasitol., 1919, XI, 127. Chatterjee (Philippine Jour. Sc., 1920, XVII, 385) describes an atypical ameba, which he calls *Endamaba paradysenteria* sp. nov., which caused fatal dysenteric lesions and differed in certain respects from *Endamaba histolytica*. See also, Warthin., Jour. Inf. Dis., 1922, XXX, 550; Kofoid and Swezy, Jour. Am. Med. Assoc., 1922, LXXVIII, 1602; Kofoid, Boyers and Swezy, Ibid., 1604.

The more recent the case the more numerous are these parasites; (3) they are deposited in the dysenteric ulcers and tend to pass into the deeper tissue appearing as true tissue parasites; (4) they frequently deposit themselves in the liver causing abscesses which contain these organisms in large numbers and practically no other infectious material; (5) by injection of amebæ-containing feces into the large intestine of animals typical amebic dysentery may be caused. It is to be borne in mind that symbiosis with bacteria is apparently necessary for the development of amœbæ.

The *Endameba histolytica* is an actively motile, roundish, pear-shaped, oval, or irregular unicellular organism having an endosarc which is typically granular and may contain leucocytes, red blood-cells, bacteria, particles of food, or pigments which the parasite has ingested, and shows the clear hyalin ectosarc, which is, perhaps, best seen in the pseudopoda. The pseudopoda are the typical motile portions of the parasites, these projections being thrown out from any point of the periphery, the protoplasm seeming to flow into them and drawing the animal after it. The parasite may not move, but will change its external appearance by throwing out these pseudopoda in various directions. The nucleus is a homogeneous, little refractile, chromatin-poor, spherical mass, about 6 microns in diameter (the diameter of the organism itself ranging between 10 and 50 microns, the average being 35). This nucleus is not always clearly visible, appearing much more frequently in the animal killed by corrosive sublimate. In the granular endosarc one frequently sees several vacuoles which may or may not pulsate, the general opinion being that pulsation is absent, although change in shape is frequent.¹

As Craig² states, "It is now well known that it is not the patient sick with acute symptoms of endamebic dysentery who is the greatest source of danger to the uninfected but the apparently healthy individual, or one who has recovered from the acute attack, but who is carrying cysts of *Endameba histolytica* in his stools. The Cystic stage of *Endameba histolytica* is the chief agent of infection in this disease, for while the vegetative motile forms may produce infection when ingested, the evidence is conclusive that in both man and animals the ingestion of cysts is followed by infection in the vast majority of instances. . . . Cysts of *Endameba histolytica* occur in chronic infections; in cases in which treatment has not resulted in the destruction of all the endamebæ; and in apparently healthy individuals who have never presented symptoms of dysentery. For the latter class the name 'contact carrier' is suitable, while for those individuals who develop

¹ If one wishes to stain such preparations, the method of Darling (Jour. Am. Med. Assn., 1912, LIX, 292, and Science, 1913, XXXVII, 58) is reliable. Stain with Wright's stain in the usual way and follow this with Giemsa's stain until the film has a purple cast. Then plunge this into 60 per cent. alcohol to which 10 to 20 drops of aqua ammoniæ have been added. Differentiate in this way until the film has a violet color. Dry and examine. See, also, Craig, War Dept., Office of Surgeon General, Bull. 1, 1913, 11; Donaldson, Lancet, 1917, I, 571, advocates the use of a mixture of equal parts of (1) five per cent. aqueous solution of potassium iodid saturated with iodine, to which an equal volume of ether is added, and (2) a saturated aqueous solution of rubin S or of eosin. A loopful of feces mixed with a few loopfuls of the above mixture is placed on a glass slide and covered with a clean cover-glass. The cysts stand out as brilliant yellow or greenish yellow spheres with a sharply defined outline.

² Mil. Surg., 1917, XL, 286 and 423.

cysts during convalescence from an acute attack of endamebic dysentery the name 'convalescent carrier' is used."

On account of the great source of danger from these carriers of amebic infection and, also, to the fact that such carriers harbor these cysts for many months and possibly years, it is important that the stools be searched carefully for these cysts in every suspected case. According to Kofoid, Kornhauser and Swezy,¹ the cysts of *endameba histolytica* are distinguished by their glassy, refractive, irregularly vacuolated or (in small cysts) almost homogeneous cytoplasm, with distinct nuclei with central granule and heavy rim never exceeding four in number, with relatively abundant mononuclears and glycogen, when present, diffusely distributed. These cysts are usually spherical and fall into three general groups according to size, the largest being from 12 to 14 microns in diameter and the smallest from 7 to 8 microns. The cyst wall is thin and rather easily penetrated by the Donaldson stain (see page 147) and by hematoxylin stains. With the Donaldson stain the cytoplasm is first a bluish gray which changes to a yellow and then to a pink color, which gradually deepens to red. The cytoplasm is unevenly vacuolated and is very finely granular but the granules are not so evenly distributed as in *Endameba coli*. Chromatoid rods are found in a majority of the cysts. Nuclei one to four in number. The chromatin appears to be evenly distributed on the nuclear membrane, which seems to be thicker than in *Endameba coli*, and there is present a distinct central granule, visible under proper focus as a clearly defined central dot.²

The stools of this amebic dysentery are thin and watery, show an alkaline reaction, and have a peculiar lime-like odor. Much mucus, blood, and occasionally many pus-cells are found for which reason the mucus should be selected for examination in case these organisms are suspected.³ Frequently one may obtain better specimens for examination by examining mucus which is obtained with a rectal tube.

¹ Arch. Int. Med., 1919, XXIV, 35. Root (Am. Jour. Hyg., 1921, I, 131) has shown that flies may act as distributors of *endameba*, the cysts of the parasite surviving for some time in the bodies of flies. Boeck (Ibid., 365) has shown the resistance of protozoan cysts to high temperatures, the thermal death point of cysts of *endameba histolytica* being 68 C and that of the *endameba coli* being 76 C.

² See Fischer, Deutsch. Arch. f. klin. Med., 1915, CXVIII, 129; Ravaut and Krolunsky, Presse Med., 1916, XXIV, 169 and 289; Sanford, Jour. A. M. A., 1916, LXVII, 1923; Cropper and Rowe, Lancet, 1917, I, 179; Boeck, Univ. Calif. Pub. Zool., 1917, XVIII, 145; Carles and Barthelmy, C. R., soc. biol. de Paris, 1917, LXXX, 402; Mathis and Mercier, Presse Med., 1917, XXV, 114; Dobell and Jepps, Brit. Med. Jour., 1917, I, 607; Walker and Emrich, Jour. A. M. A., 1917, LXVIII, 1456; Ravaut, Presse Med., 1917, XXV, 81; Wenyon and O'Connor, Jour. Royal Army Med. Corps, 1917, XXVIII, 686; Shimura, Jour. Exper. Med., 1918, XXVIII, 415; Mathews, Ann. Trop. Med. and Parasitol., 1918, XII, 17; Smith, Ibid., 27; Cort and McDonald, Jour. Infect. Dis., 1919, XXV, 501; Haig, Lancet, 1919, II, 823; Cragg, Indian Jour. Med. Res., 1919, VI, 462; Kofoid, Kornhauser, and Plate, Jour. Am. Med. Assoc., 1919, LXXII, 1721; Lyons, Southern Med. Jour., 1920, XIII, 4; Jels, Ibid., 23; Lynch, Jour. Am. Med. Assoc., 1920, LXXV, 5; Yoshida, Jour. Exp. Med., 1920, XXXII, 357; Kofoid and Swezy, Am. Jour. Trop. Med., 1921, I, 41; Thomson and Robertson, Proc. Roy. Soc. Med., Sect. Trop. Dis. and Parasitol., 1921, XIV, 33; Scott, Ann. Trop. Med. and Parasitol., 1921, XV, 133 and 149; Craig, Jour. Am. Med. Assoc., 1921, LXXVII, 827; Glaser, Jour. Parasitol., 1921, VIII, 1.

³ Musgrave and Clegg advise the administration of a saline cathartic and the later examination of the fluid portion of the stool.

(β) **Endamoeba coli** (*amoeba coli* Lösch).

This parasite varies in size between 10 and 15 microns. The hyaline protoplasm of the pseudopoda is not distinctly differentiated from the ectoplasm. It is opaque, gray in color, and its nucleus is sharply defined, being characterized by its richness in chromatin. The movements of this organism are not as rapid as those of the *endamoeba histolytica*, which does not show as active phagocytic power. According to Craig, about 65 per cent. of normal persons show these non-pathogenic *endamoeba coli* in the feces.

Cysts of *Endameba coli* are generally spherical and range from 14 to 22 microns in diameter. Cyst wall is thicker than that of the *histolytica* and is clearly defined. Rather easily attacked by the iodine-eosin stain of Donaldson but acts irregularly toward iron hematoxylin. Cytoplasm is more coarsely but more uniformly granular than that of the *histolytica*. Glycogen rarely present except in cysts having fewer than eight nuclei. Chromatoid bodies rarely seen. Nuclei generally eight in number, but large cysts may contain sixteen. These appear clear and definite in the iodine-eosin stain. Each nucleus has a central granule and peripheral chromatin situated in the nuclear membrane in uneven masses, giving, at sharp focus, a rather ragged inner edge to the refractive ring of chromatin material.¹ The nucleus of *Endameba nana* shows no central granule and the peripheral chromatin is massed in a single large clump at one point on the nuclear membrane.

(b) **Sporozoa.**

Coccidium hominis (*coccidium perforans*; *cystospermium hominis*).

This organism appears in the feces as an oval or spherical parasite about 22 microns long and showing a thin periphery. A large number of nuclei are usually observed.

The infection with these organisms seems to arise from rabbits in whose intestines these parasites develop in large numbers.

(c) **Flagellates.**

(α) **Trichomonas intestinalis.**

This organism was first studied by Marchand and Zunker and later elaborated by Grassi, Roos, and Janowski. It is probably identical with the one known as *trichomonas vaginalis* which may live in the vagina, the urethra, large and small intestine, the stomach, and may be found in the sputum. Various forms have been described as being found in the intestine, but they are in all probability the same organism; among these we find *protoryxomyces coprinarius*, *monocercomonas hominis*, *cimænomonas hominis*, *trichomonas hominis*, *cercomonas coli hominis*, and *cercomonas seu Bodo urinaris*.

This is a colorless protozoon of a pyriform or spindle shape, rounded in front and bearing three flagella which are apt to be merged at the base and easily lost, the posterior end pointed but not bearing a flagellum. It is from 20 to 25 microns in length and 8 to 12 broad. Along the body, start-



FIG. 26.—*Trichomonas intestinalis*. (Tyson.)

¹ See Kofoid, Kornhauser and Swezy, *loc. cit.*

ing from the base of the flagella, runs an undulating membrane in a somewhat spiral manner to the posterior end. It has a finely granular cytoplasm and at its anterior end a vesicular nucleus, behind which one or more non-pulsating vacuoles may be seen. At times this organism may be observed to assume an ameboid form, the movements of the flagella having then ceased and projections resembling pseudopoda being observed.¹

(β) *Cercomonas hominis*.

This organism was first studied by Davine and has been found by many other workers. It is known under the names of *cercomonas intestinalis*, *monocercomonas hominis*, *cimænomonas hominis*. The adult organism is a small, colorless, pyriform parasite, with round anterior end provided with one long flagellum and a pointed posterior end. It is 8 to 10 microns long and has no undulatory membrane, as has the *trichomonas intestinalis*.

(γ) *Megastoma entericum*.

This organism was first found by Lambl in the feces of children. It is known under several names among which are *Lambliia intestinalis*, *hexamitus duodenalis*, *dimorphous muris*,



FIG. 27.—*Cercomonas hominis*; A, larger and B, smaller varieties. (Tyson.)

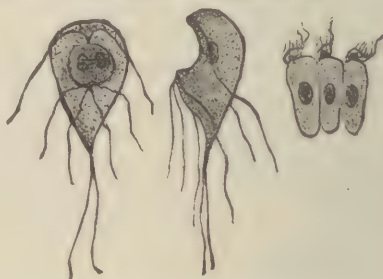


FIG. 28.—*Megastoma entericum*, showing disk-surface and lateral views in larger figures, and three epithelial cells with attached examples to the right. (Tyson.)

megastoma intestinale and *giardia intestinalis*. This parasite is a colorless pear-shaped protozoon with a rounded anterior end and a pointed posterior end bearing a pair of flagella. The anterior end has one side concave with a raised border or lip, one pair of flagella, arising at the anterior border of this disk-like concavity, and two pairs together from its posterior margin. The cytoplasm is finely granular and the dumb-bell shaped nucleus is anteriorly about the middle level of the concavity. Vacuoles are absent and solid inclusions are never observed. The length of these organisms is from 15 to 16.5 microns, while the width is from 10 to 12.5 microns. The number of these organisms found in the feces may be very large. The surest points for their diagnosis seem to be the concavity and the dumb-bell shaped nucleus. The source of infection for man is the drinking of infected water.²

¹ See Castellani, Jour. Trop. Med. and Hyg., 1914, XVII, 65; Lynch, New York Med. Jour., 1915, CI, 886; Jour. Parasitol., 1916, III, 28; Rhamy and Metts, Jour. A. M. A., 1916, LXVI, 1190; Chase and Tasker, Ibid., 1917, LXVIII, 1528; Chalmers and Pekkola, Jour. Trop. Med., and Hyg., 1916, XIX, 142, report the finding of a new intestinal flagellate, ootomitus hominis, with six anterior flagella.

² See Kennedy and Rosewarne, Lancet, 1916, I, 1163; Fantham and Porter, Brit. Med. Jour., 1916, II, 139; Logan and Sanford, Jour. Lab. and Clin. Med., 1917, XI, 618; Mantovani, Gazz. d. osp., 1919, XL, 66; Labbe, Presse Med., 1919, XXVII, 161; Carles, Jour. de med. de Bordeaux., 1919, XC, 187; Kofoid, Kornhauser and Plate, Jour. Am. Med. Assoc., 1919, LXXII, 1721; Cade and Hollande, Arch. de mal. de l'app. digestif., 1919, X, 193; Deglos, Lyon Med., 1920, CXXIX, 434; Cress, Med. Record, 1920, XCVIII, 143; Maxcy, Bull. Johns Hopk. Hosp., 1921, XXXII, 166; McGill, Jour. Am. Med. Assoc., 1922, LXXVIII, 179.

(d) **Infusoria.**

Balantidium coli (paramœcium coli). This parasite is colorless, ovoid in shape, 70 to 100 microns long and 50 to 70 microns broad, having a finely granular cytoplasm containing fragments taken from the intestinal material, and a clear ectoplasm showing numerous longitudinal striations. It is covered completely with actively motile cilia which are more dense about the funnel-shaped mouth which extends about one-fourth the length of the body. The nucleus is kidney shaped and is usually accompanied by one or more accessory nuclei, while two or more contractile vacuoles are seen which pulsate to a slight extent.

This parasite has been found in connection with various types of diarrheal affection and also in persons entirely free from intestinal symptoms. It may be present in the stools in very large numbers, being found especially in the colon, but in severe cases also in the jejunum. The hog is the most common host of this parasite, which at times shows marked pathogenicity (see Bowman¹).

(2) **Entozoa (Enthelmintha).**(A) **Platodes (Flat Worms).**(a) **Cestodes (Tape-worms).**

The cestodes are naked, flat worms of elongated ribbon shape, endoparasitic, at least in their adult stage, and in many instances in all stages, without a digestive canal, and always more or less distinctly divided into segments. The entire parasite, or *strobile*, may be looked upon as a colony of individuals united in ribbon fashion from their mode of origin, for convenience in their development and functional performance; the various segments being derived by a process of constriction from the originally acquired parasite, which is spoken of as the *head*, *nurse*, or *scolex* of the strobile. A characteristic of the cestodes is the differentiation of two developmental stages: the first, *cysticercus stage*, in which the connective tissue or parenchymatous organs are invaded, and, secondly, the development of the sexually mature animal in the intestine. The scolex obtains entrance as a larva into the intestinal tract of the host, becomes attached by a special fixation apparatus to the mucous membrane, and there develops into the adult parasite, forming the anterior extremity of the strobile in the developed worm. The head is usually a very small and inconspicuous object of globular, pyriform or club shape with a short posterior extension, spoken of as the *neck*. In the middle of the frontal face of the head there is often a small prominence, known as the *rostellum*, about which may be arranged in one or more rows, as one of the means of fixation of the parasite, small hooklets as a crown. As more constant means of fixation the head is provided with two or four suckers, rounded or linear depressions with more or less definite lips. Back of the head by a process

¹ Jour. Am. Med. Assn., 1911, LVII, 1814. See, also, Hinkelmann (New York Med. Jour., 1915, CI, 200), who finds these parasites present in water supplies. The same author discusses the cultivation and reproduction of this parasite in N. Y. Med. Jour., 1919, CIX, 235. See Logan, Am. Jour. Med. Sc., 1921, CLXII, 668.

of constriction from the neck, the segments, also known as links or *proglottids*, arise, the newest form always being placed between the neck and the next older link. Thus the older segments are always separated more and more from the head by each newly formed proglottid, each as it grows older and recedes further from the neck developing in size; the length of the strobile being thus dependent upon the two factors, growth of the individual length and the new formation of segments. These new segments as they are first formed are usually very short and proportionately broader, but as they increase in size with age they generally enlarge especially in their long diameter, and come to be more or less square. The number of these links may vary from three or four to several thousands, the length of some worms being 100 or more feet. The structure of each link and hence of the whole strobile includes

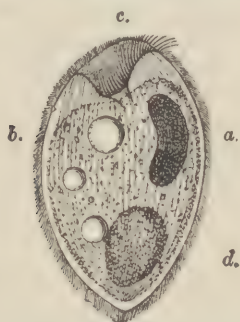


FIG. 29.—*Balantidium coli*:
a, Nucleus; *b*, vacuoles;
c, cytostome, with pit and
peristome; *d*, ingested
material. (Tyson after
Leuckart.)



FIG. 30.—Head and neck, and
ovum $\times 300$, of *tania solium*.
Embryophore surrounded by
vitellus. (Tyson after Gould.)

an interior or matrix of an indeterminate connective reticular material, from which the various organs appear to develop and in which they are imbedded; over which are to be recognized exteriorly a delicate cuticle and beneath the latter, two layers of so-called muscle, the outer layer longitudinal and the inner transverse and circular.¹

Aside from the common parts the various links may be looked upon as individuals. There is no digestive canal, all nutrition being obtained by the parasite from absorption of dissolved material from the fluids in the infested intestine. The only really highly organized parts are the generative organs, each link containing both male and female organs. The terminal links are the ones containing the most ova, while the links nearest the head are usually but partially developed. In their development the ova fill the canal of the oviduct more and more often causing the appearance of side pockets of more or less branching character. The terminal ripe links either actually containing the ova or, after discharge of more or less of the original number, are apt to become separated from the strobile and be carried with the fecal matter from the intestine. Either with or without intermediate development the embryo is in some way, by water or in solids, carried into the alimentary canal of a

¹ See Douthitt, Ill. Biological Monographs, 1915, I, No. 3.

second host. Arrived in this situation, both by its own activity and by passive convection by blood and lymph streams, the embryo penetrates the intestinal wall and becomes deposited in one or other situation as a larva, bladder-worm, or *cysticercus*. This larval or cysticercus form is surrounded by a delicate connective tissue outer wall, derived from the host by a process of reactive inflammation, within which lies the true bladder-worm. This is essentially the head of the future parasite. This cysticercus, after remaining a variable period in the tissues, is devoured with the flesh of its host by a third (definitive) host.

(a) **Tæniidæ.**

(1) ***Tænia solium*.**

This parasite, which is derived from infection through the cysticercus cellulosa of pork, has been also named *tænia cucurbitina*, *tænia dentata*, *cystotænia solium*, and *pork tape-worm*. The average length of the strobile is 2 to 3 meters, occasionally reaching twice this measurement. The head is somewhat spherical or slightly tetragonal from the four rather prominent cup-like suckers with thick lips. The head varies from $\frac{1}{2}$ to 1 mm. in diameter, while the suckers range from $\frac{1}{4}$ to $\frac{1}{2}$ mm. in diameter. It is provided with a short, thick, rostellum bearing a double crown of hooklets, usually 28 in number. The neck is thin, about 3 cm. in length and is unsegmented. The proglottids number between 800 and 900, the fully grown and ripe segments measuring from 9 to 12 mm. long and 5 to 6 mm. broad. The uterus consists of a large, median, longitudinal trunk with from 7 to 10 coarsely dendritic branches on each side. The ova are round or oval, the shell very thin but surrounded by an embryonic layer which is thick and shows distinct radiating lines. These eggs are usually of a brownish color and may show on their interior the hooklets of the embryo.

This parasite, in its adult stage is practically limited to the small intestine of man, while the larval form has been found in swine, monkeys, dogs, etc. It has been shown that careful cooking and prolonged and thorough salting and drying of the meat will destroy the vitality of the cysticercus cellulosa. It is, therefore, plain that any meat should be more or less thoroughly cooked before being eaten.

(2) ***Tænia saginata*.**

This parasite¹ is the *beef tape-worm* and is also known as the *tænia medio-canellata*, *tænia inermis*, and *tænia dentata*. The adult worm varies from 3 to 8 meters in length, has a head from 1 to 2 mm. in diameter, tetragonal in shape without hooklets or rostellum, with four cup-shaped suckers each 0.8 mm. in diameter and placed at the corner of the frontal face. The ripe segments are from 18 to 20 mm. long and 5 to 7 mm. broad. The uterus shows a distinct median longitudinal trunk with 20 to 35 lateral single or dichotomously branching and slender diverticula. The eggs are spherical with a

¹ See Hodson, *Trop. Med. & Hyg.*, 1921, XXIV, 244.

thin shell surrounded by a thick radially striated embryonic shell. These eggs are from 30 to 40 microns long by 20 to 30 microns wide.

(3) *Tænia cucumerina*.

Infection with this parasite is relatively rare in the United States. It is found almost exclusively in children, the infection occurring through dogs and cats, the larval form of the parasites being found in the body lice and fleas.¹ This organism has other synonyms, among them being *tænia canina*, *tænia moniliformis*, *tænia elliptica*, *dipylidium caninum*, and *dipylidium cucumerinum*. The parasite is from 15 to 35 cm. in length. The head is small, rhomboidal, with a clavate rostellum surrounded by three or four crowns of hooklets (48 to 60 in number), suckers rather large with radially marked borders, and neck very short. The segments are from 80 to 150 in number, the older ones being 8 to 11 mm. in length and 1 to 3 mm. in breadth and often showing a reddish-brown color. The links frequently swell out in the middle so that the parasite has an appearance not unlike a chain of beads.



FIG. 31.—Head and neck of *tænia saginata*: A, retracted; B, extended. (Tyson after Gould.)



FIG. 32.—*Tænia cucumerina*. (Tyson after Leuckart.)

A single uterus is common to the two oviducts, consisting of a network of tubes in which the ova lie in groups, filling small saccules, each containing 10 or 15 ova and surrounded by a reddish material which gives the color to the worm. The ova are spherical, 43 to 50 microns in diameter, and have a double wall. Within the wall one observes an embryo armed with hooklets.

(4) *Tænia nana*.

This worm is known as the "dwarf tape-worm" and is perhaps best known in Italy and Southern Europe, although it has been found in many cases in the



FIG. 33.—*Tænia nana*. $\times 10$. (Tyson after Gould.)

eastern and southern portions of the United States.² It has been called the *tænia ægyptica*, *hymenolepis nana*, *hymenolepis murina*, and *diplacanthus nana*.

¹ See Lins, Wien, klin. Wchnschr., 1911, XXIV, 1595; Ancona, Rif. Med., 1916, XXXII, 652.

² See Greil, Am. Jour. Dis. Child., 1915, X, 363; De Buys and Dwyer, Am. Jour. Dis. Child., 1910, XVIII, 269; Kofoid, Kornhauser and Plate, Jour. Am. Med. Assoc., 1910, LXXII, 1721; Goldman, Arch. Int. Med., 1920, XXVI, 373; Am. Jour. Trop. Med., 1921, I, 109; Chandler, Jour. Am. Med. Assoc., 1922, LXXVIII, 636.

The infection with the ova is probably through the use of unfiltered water tainted with human or murine dejecta. It is most frequently seen in children, and inhabits the ileum, usually from the middle toward the ileocecal valve.

The parasite is from 10 to 15 mm. in length and from 0.5 to 0.7 mm. broad, is provided with a subglobular head measuring 0.2 to 0.3 mm. in transverse diameter, shows four large rounded suckers and a large rostellum retractile into an infundibulum. The rostellum is surrounded by a single row of characteristic hooklets, 24 to 30 in number and 14 to 18 microns in length. The neck is rather long and slender, being followed by about 150 small proglottids, which are broader than long (0.4 to 0.9 mm. broad by 0.14 to 0.3 long).

The ova are characteristic. They are round or oval in shape, 32 to 36 by 42 to 56 microns in size, and have two distinct membranes. At each pole of the inner membrane is seen a small protuberance from which springs a number of clear refractile threads, which are distributed in a waving fashion through the substance intermediate to the outer and inner walls.



FIG. 34.—Head and neck of *tænia diminuta*. (Tyson after Braun.)

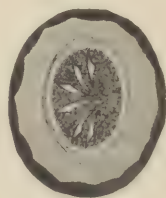


FIG. 35.—Ovum of *tænia diminuta*. (Tyson after Braun.)

(5). *Tænia diminuta*.

SYNONYMS.—*Hymenolepis diminuta*; *hymenolepis flavopunctata*; *tænia leptcephala*; *tænia flavopunctata*; *tænia minima*; and *tænia varerina*.

The parasite is 10 to 60 mm. long, head small, globular, with four globose suckers situated close to apex, rostellum small, pyriform, and devoid of hooklets. The segments number 800 to 1300 and are broader than long. Gravid uterus nearly fills the segments, showing as transverse line when not ripe.

The ova are round or slightly oval, yellowish in color, double-walled, inner wall showing slight protuberances at poles, a layer of albuminous material being seen between the walls.

(6). *Tænia echinococcus*.

This parasite in its adult stage is met with in the upper part of the small intestine of dogs, wolves, and jackals. The larval form, known as the *hydatid cyst*, is found in man, although more frequently in the ox, hog, horse, dog, cat, rabbit, etc. This disease is rare in America, and is acquired through association with the dog more frequently than in other ways. The most common seat of hydatid disease is in the liver.¹

The parasite is 2 to 5 mm. long, has a small subglobular head measuring

¹ See Phillips (Jour. Am. Med. Assn., 1913, LXI, 1981), who reports such a cyst in the pancreas. Delgado, Cronica Medica, 1916, XXXIII, 341; Llambias, Semana Medica, 1916, XXIII, 359.

0.3 mm. in transverse diameter and bearing a rostellum with a double row of very characteristic hooklets (28 to 50 in number) and four prominent cup-shaped suckers. The neck is short and rather thick; proglottids three or four in number, the last of which is usually longer than the rest of the worm put together. Uterus consists of a thick longitudinal median trunk with a few short lateral branches.

Ova spheroidal with thin radially striated shells and containing a granular hexacanthus embryo. Length of ova 30 to 60 microns, transverse diameter 25 to 30 microns.

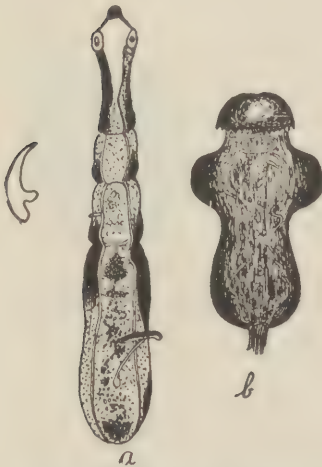


FIG. 36.—*Tænia echinococcus*: a, Adult; b, head from echinococcus cyst. On left a detached hooklet, as seen in fluid from cyst. (Tyson after Coplin and Bevan.)

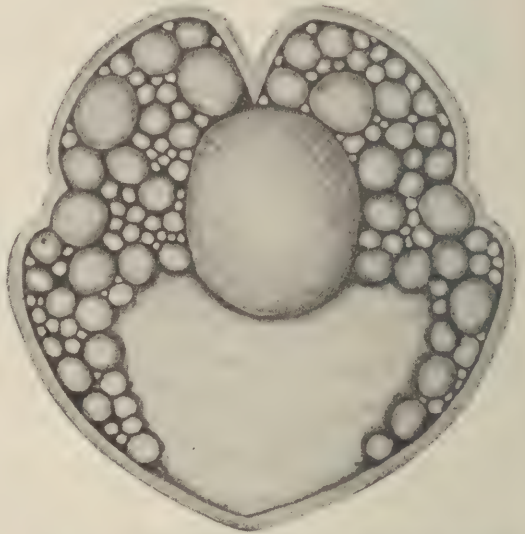


FIG. 37.—Hydatid cyst, showing daughter cysts. In the lower part of field is a whitish mass containing parts of the walls of ruptured daughter cysts. The thick wall of the mother cyst is well shown. From liver of man, $\times \frac{2}{3}$. (Coplin.)

(b). *Bothriocephaloidea*.

(1). *Bothriocephalus latus*.

SYNONYMS.—*Dibothriocephalus latus*; *tænia lata*; *dibothrium latum*; *diphyllbothrium latum*; *bothriocephalus latissimus*; fish tape-worm.

This parasite is most commonly met in the human intestine, but may be found in dogs and cats. It is most common in central Europe and in the maritime countries of Europe, British Islands, and Japan. The examples found in America occur in foreigners, as a rule. The ova, which are usually in large numbers in the feces, require for their future development immersion in water. The liberated embryo is then taken up by freshwater fish and conveyed to man.

Strobile 2 to 10 meters (20 in a few cases) in length, marked in ripe segments by brownish central rosette (uterus with ova). Head elongated, almond shaped, 2 to 5 mm. long and 0.7 mm. transversely, with two lateral grooves or bothridia as suckers. Neck variable according to degree of contraction. The segments number 3,000 to 4,000 and begin about 50 cm. from

the head. The anterior links are poorly defined, in their growth increasing slowly in length but markedly in breadth. The ripe links measure 2 to 4 mm. in length and 10 to 12 in width, with opaque brownish rosettes in the middle line. Uterus formed of a number of plicated tubes in the form of a rosette.

The ova are brownish in color, ellipsoidal in shape, 68 to 71 microns in length and 44 to 45 in transverse diameter, have a thin shell, and a lid which may be opened or closed.

The contents of the ova are coarsely granular or mulberry-like.¹

Infection with this worm is not always single, as high as 100 worms having been reported in the same individual. Many cases of infection with this parasite are associated with a high-grade anemia, which is distinguishable from pernicious anemia only by the effects of removal of the parasite.

(2) *Dibothriocephalus cordatus*.

This is a tape-worm of the same genus as the above and is parasitic in seals, being transmitted from them to man. It varies in length from 80 to 115 cm. Proglottids about 600, 7 to 8 mm. broad. The head is heart-shaped, 2 mm. long and broad.

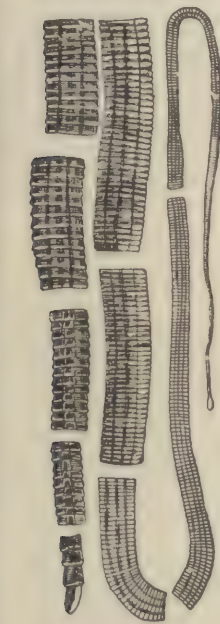


FIG. 38.—*Bothriocephalus latus*. (Tyson after Leuckart.)



FIG. 39.—*Dibothriocephalus cordatus*: adult. (Tyson after Leuckart.)

The ova are similar to those of the *latus*, but are a little larger in size.

(3) *Bothriocephalus* sp. Ijima et Kurimoto.

SYNONYMS.—*Diplogonoporus grandis*; *Krabbea grandis*.

Strobile measures up to 10 meters in length. Proglottids short and broad, head, neck, and number of segments unknown. Uterus rosette-shaped with several loops on each side. Ova brownish, operculated, oval, 63 microns in length and 48 to 50 in width. Intermediate host unknown, probably fish.

(β) Trematodes (Fluke-worms).

The various forms of distoma, which belong to this class, are more properly hepatic parasites, although they and their ova may at times appear

¹ See Rubenstone, N. Y. Med. Jour., 1916, CIV, 599; Magath, Jour. A. M. A., 1919, LXXIII, 85; Riley, Ibid., 1186; Becker, Finska Läkär. Handl., 1920, LXII, 240; Nickerson, Jour. Am. Med. Assoc., 1920, LXXIV, 457; Lyon, Ibid., 655; Low and O'Driscoll, Brit. Med. Jour., 1921, I, 118; Calvin, Jour. Am. Med. Assoc., 1922, LXXVIII, 84; Wallace and Grant, Ibid., 1050.



FIG. 40.—*Ascaris lumbricoides*: to left, male in lateral aspect; to right, female, ventral aspect, natural size. (Tyson after Railliet.)

in the intestines and feces. A detailed discussion of these parasites will be taken up in the chapter on Parasites.

(B) Nematodes (Round Worms).

The nematode worms are unsegmented, elongate, circular or nearly so in their transverse section, cylindrical or more or less delicately fusiform and tapering toward each end. They are with but few exceptions parasitic and include many important examples, which are parasitic in man. Intermediate hosts, so essential for the intermediate development of the flukes and tapeworms, are practically absent in the nematodes.

(a) *Ascaridæ*.

(1) *Ascaris lumbricoides*.

This is the common round worm or maw-worm seen so frequently in children. The number in a single host is usually small, but may be very large. Its habitat is the small intestine, but the eggs may occur in the vomitus as well as in the feces.

The male worm is whitish to reddish-yellow in color; 15 to 17 cm. long, 3 to 3.5 mm. thick; elongate, fusiform; cuticle finely ringed; oral orifice terminal with three lips (one dorsal and the other two meeting in the median ventral line), each with fine denticulations on margins; at base of superior lip two papillæ, one only at base of other two lips; posterior end terminating conically, curved ventrally, with two slightly curved, short, equal spicules projecting from the subventral cloaca; 70 to 75 papillæ on the ventral face of posterior end, of which seven pairs are postanal.

The female parasite is 20 to 25 cm. long, 5 to 5.5 mm. thick; anterior end and general appearance as in male; posterior end tapering, ending in a conical, pointed, straight tail, vulva at level of first third of body length (in a slightly depressed annular band); anus subterminal.

The ova are elliptical in shape, 50 to 75 microns long and 40 to 58 microns broad; shell thick and transparent; stained yellowish with fecal material when in feces, but colorless in uterus; protoplasm unsegmented and

coarsely granular; covered with a mammilated albuminous envelope which may be lost.¹

(2) *Ascaris mystax*.

SYNONYMS.—*Ascaris canis*; *ascaris lumbricus canis*; *ascaris teres*; *ascaris caniculæ*; *ascaris cati*; *ascaris tricuspidata*; *ascaris felis*; *ascaris weneri*; *ascaris marginata*; *ascaris alata*; and *fusaria mystax*.

The male parasite is whitish or slightly brownish; 40 to 60 mm. long, 1 mm. thick; anterior end usually curved, with lateral cuticular expansions making the end look somewhat arrow-like; mouth terminal with three nearly equal lips with denticulate margins; at base of superior lip two papillæ, on inferior lip one ordinary and two minute papillæ; posterior end curled and with lateral cuticular alar expansions, and on each side of cloacal aperture 26 papillæ, of which five are postanal.

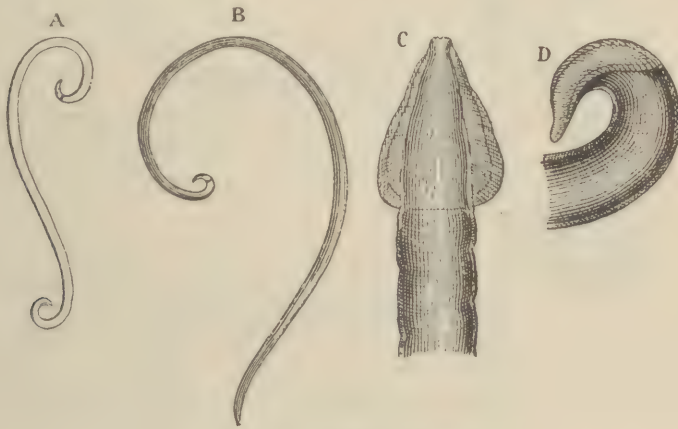


FIG. 41.—*Ascaris mystax*.

A, Male; B, female; C, anterior extremity, enlarged and shown from dorsum to exhibit the lateral wing-like cuticular expansions; D, same showing in profile. (Tyson after Railliet.)

The female worm is 120 to 180 mm. long; anterior end and general appearance as in male; posterior end straight, terminating obtusely; vulva at anterior fourth of body length; anus subterminal.

The ova are almost spherical; 68 to 72 microns in diameter; shell thin with thin albuminous envelope showing an alveolated surface.²

(3) *Oxyuris vermicularis*.

SYNONYMS.—*Ascaris vermicularis*; *fusaria vermicularis*; *ascaris græcorum*; *pin-worm*; *threadworm*; *seat-worm*.

The male is whitish in color; 3 to 5 mm. long, 0.3 to 0.4 mm. thick; cuticle transversely striated and at head end showing a vesicular swelling along the dorsal and ventral median lines; lateral lines distinct; mouth

¹ See Foster, Jour. Parasitol., 1914, I, 31; Perret and Simon, Jour. A. M. A., 1917, LXVIII, 244; Ransom, Ibid., 1919, LXXIII, 1210; Tyau, National Med. Jour. of China, 1920, VI, 21; Pentland, Practitioner, 1920, CIV, 313; Pillay, Indian Med. Gaz., 1921, LVI, 100; Palermo, Brazil Med., 1921, XXXV, 171; Pessoa, Jour. Am. Med. Assoc., 1922, LXXVIII, 1294.

² See Beisele, Münch. med. Wchnschr., 1911, LVIII, 2391; Flury, Arch. f. exper. Path. u. Pharmacol., 1913, LXVII, 294.

terminal, with three retractile lips; esophagus with distinct bulb; posterior end conical, curved ventrally, with six pairs of papillæ and slight cuticular expansion on each side; one spicule hooked at free end.



FIG. 42.—*Oxyuris vermicularis*: to left, female; to right, male (considerably enlarged); A, anus; O, mouth; v, vulva. (Tyson after Braun.)

The female is 10 mm. long, 0.6 mm. thick; anterior end and general appearance as in male; posterior end straight, extended to a long mucronate tail; vulva at anterior third of body length.

The ova are oval, flattened on one side with a characteristic asymmetry, 50 microns long and 16 to 20 broad; shell thin; colorless.

This parasite inhabits the rectum and colon, but it may travel even into the stomach. The ova are rarely found in the feces, except in the mucus or about the anus.¹

(b) *Angiostomidæ*.

Strongyloides intestinalis.

SYNONYMS.—*Anguillula intestinalis et stercoralis*; *leptodera intestinalis et stercoralis*; *pseudorhabditis stercoralis*; *rhabdonema strongyloides*; *rhabdonema intestinalis*.

This organism is found in two different forms: the first diöic and free, the second parasitic, as parthogenetic females. The parasitic form lives in the upper intestinal tract of man; is 2.5 mm. long, cylindrical, with pointed tail end, cuticle smooth; mouth simple with four lips; long, slender, cylindrical esophagus reaching one-fourth of the length of the worm; anus close to tail; vulva at posterior third, containing yellowish-green oval ova, 50 to 58 microns long and 30 to 34 microns broad. The larvæ develop in the intestine and are passed in the fecal material. These larvæ are at first from 200 to 240 microns in length, but increase to two or three times this length. The larvæ differ essentially from the parent in having a rhabditiform esophagus.

In the discharged feces at about 30°C. these develop with one moulting of the cuticle to a free-living generation with separate sexes.

In this free sexual generation the worms are smooth, cylindrical, and tapering, with pointed tail-ends; the mouth is the same as in the parasitic form; esophagus rhabditiform with its anterior portion long and with the posterior pyriform and containing a Y-shaped chitinous armature; anus at base of tail; male with tail curved and two spicules, body length 0.7 mm.; female

¹ See Crowell and Hammack, *Philippine Jour. Sc.*, 1913, VIII (B), 157; Willets, *Ibid.*, 1914, IX, 233; Fracker, *Jour. Parasitol.*, 1914, I, 22; Aschoff, *Berl. klin. Wchnschr.*, 1914, LI, 1504; Suzuki, *Surg. Gyn. and Obs.*, 1915, XXI, 702.

1 mm. long, with straight pointed tail, vulva a little back of the middle; ova few, yellowish, ellipsoid, thin-shelled, 70 by 45 microns in size, sometimes hatching in the uterus. The larvæ of this generation look much as their free parents, are at first 0.22 mm. in length, but grow to 0.55 mm., then moult and assume a filariform or strongyloid character like that of the parasitic grandparent. These gain access to the intestine of a new host in an unknown manner or shortly die.

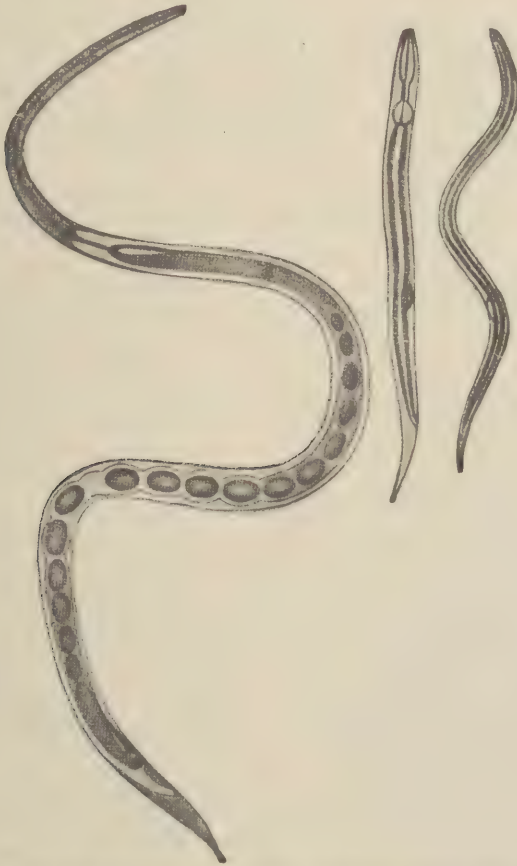


FIG. 43. —*Strongyloides intestinalis*; on the left, a gravid female from human intestine (natural size 2.5 mm.). In the middle, a rhabditiform larva from fresh fecal matter, $\times 120$; to the right, a filariform larva from culture, $\times 120$. (Tyson after Braun.)

These worms may be found throughout the upper gastrointestinal tract, especially in the duodenum and upper part of the jejunum. The time elapsing between infections with the filariform larvæ of the sexual generation and the appearance of the rhabditiform embryos of the parasitic type in the stools is between two and three weeks. The parthogenetic female types are usually called the *strongyloides intestinalis*, while the free sexual form is styled *strongyloides stercoralis*.

This form is found widely distributed in Indo-China, the East Indies,

Africa, Europe, and North and South America. The mode of transmission to the second host is probably through the means of unfiltered water or of unclean, uncooked vegetables.¹

(c) **Trichotrachelidæ.**

(1) **Trichiuris trichiura.**

SYNONYMS.—*Ascaris trichiura*; *trichocephalus trichiurus*; *trichocephalus hominis*; *trichocephalus dispar*; *trichocephalus mastigodes*; *whip-worm*.

The male worm is 35 to 45 mm. long; whitish; anterior three-fifths slender and thread-like; posterior two-fifths thicker, cylindrical, terminally rounded and curled; anus terminal; single spicule in a tubular sheath containing small spinules.

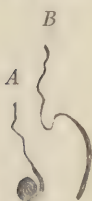


FIG. 44.—*Trichiuris trichiura*, natural size: A, Male; B, female. (Tyson.)

The female parasite is 35 to 50 mm. long; shape as in male for front and body; posterior extremity straight, bluntly pointed terminally; vulva at beginning of thick posterior portion of body.

The ova are very characteristic, brown, oval, thick-walled, with a colorless shining button-like protuberance at each pole. The eggs are 50 to 54 microns long and 23 microns wide, with an unsegmented yolk. Occasionally these eggs show variations in shades of brown, some being very much lighter than others.²

(2) **Trichinella spiralis (trichina spiralis).**

The male worm is 1.4 to 1.6 mm. long and 0.04 mm. thick; cylindrical in shape; anterior end tapering, posterior end gradually and slightly thickening and terminating in a bifid extremity with two lateral somewhat conical tail appendages; cloacal aperture between these, which form a sort of bursa; back of cloacal aperture two pairs of papillæ.

The female worm is 3 to 4 mm. long; anterior end as in the male; posterior end nearly of same thickness to tail, which is rounded; anus terminal; vulva at anterior fifth of body; viviparous.

The larvæ when born are 90 to 100 microns in length, obtuse anteriorly, posteriorly prolonged to a pointed tail; when encysted as "muscle trichinæ" the larvæ measure about 1 mm. long and 0.04 mm. in thickness, tapering anteriorly, more thick and obtuse posteriorly with complete organization as in the adult and showing the characters of the different sexes.

This parasite or its larvæ are rarely if ever found in the stools. In its adult sexual stage it infests the intestinal tract of man and a number of mammals, gives origin to a large number of larval worms, after which the adults die. The larvæ pass through the muscular wall of the intestine and are carried by the blood-current into various muscles of the host. Here they pass an

¹ Barlow, Interstate Med. Jour., 1915, XXII, 1201; Wachenheim and Bernstein, Jour. A. M. A., 1916, LXVI, 1092; Fennell, Jour. Am. Med. Assoc., 1920, LXXV, 625; Ginsburg, Ibid., 1137; Trejo, Ibid., 1921, LXXVI, 469.

² See De Buys and Dwyer, Am. Jour. Dis. Child., 1919, XVIII, 269; Hannah, Georgia Med. Assoc. Jour., 1920, IX, 69.

indefinite encysted stage, a capsule forming around them and becoming calcified.¹

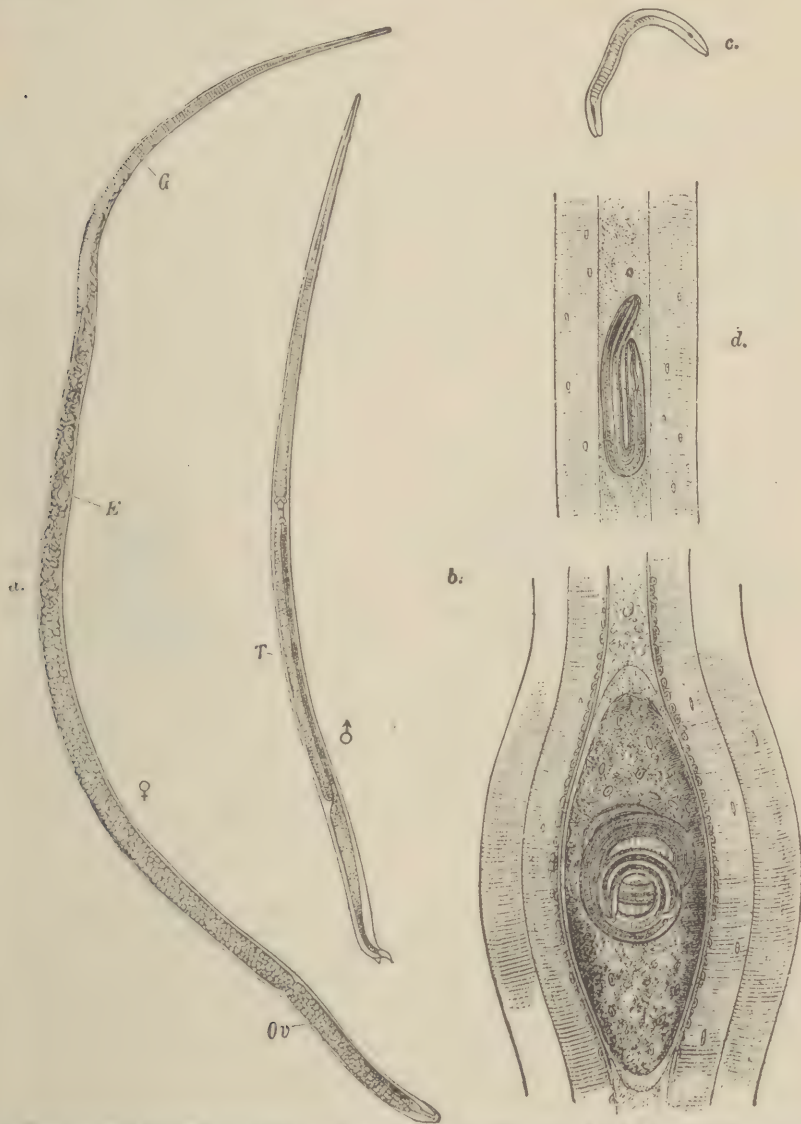


FIG. 45.—*Trichinella spiralis*: *a*, Gravid female, "intestinal trichiura;" *E*, embryos; *G*, vulva; *Ov*, ovary; *b*, adult male, "intestinal trichiura;" *T*, testicles; *C*, young larva; *d*, larva in musculature; *e*, encapsulated larva in muscle. (Tyson after Braun.)

Herrick and Janeway,² Packard,³ and Cross⁴ found the embryo in the

¹ See Flury, Arch. f. exper. Path. u. Pharmacol., 1913, LXIII, 164; Gruber, Münch. med. Wehnschr., 1914, LXI, 645; Van Cott and Lintz, Jour. Am. Med. Assn., 1914, LXII, 680; Herrick, Jour. A. M. A., 1915, LXV, 1870; Bloch, Ibid., 2140, reports the finding of the larvae in the Cerebrospinal fluid; Bloch, Ill. Med. Jour., 1916, XXIX, 369; Lintz, Jour. A. M. A., 1916, LXVI, 1856; Cummins and Carson, Ibid., 1856; Salzer, Ibid., 1916, LXVII, 579; Cummins and Carson, Ibid., 806; Stiles, Ibid., 1917, LXVIII, 685.

² Arch. Int. Med., 1909, III, 263.

³ Jour. Am. Med. Assn., 1910, LIV, 1297.

⁴ Arch. Int. Med., 1910, VI, 301.

blood, the latter in blood from an ear puncture. The blood was laked with 3 per cent. acetic acid, was centrifuged, and the sediment examined as outlined by Stäubli.¹ The chief means of infection of man with the trichinella spiralis is through the eating of insufficiently cooked pork, especially ham.

(d). **Strongylidæ.**

(1). **Uncinaria duodenalis.**

SYNONYMS.—*Ancylostoma duodenale*; *strongylus quadridentatus*; *dochimus ancylostomum*; *sclerostoma duodenale*; *strongylus duodenalis*; *dochmius duodenalis*, and *European hook-worm*.

The male parasite is whitish or blotched posteriorly with brownish when the intestine contains blood; 8 to 10 mm. long; cuticle finely striated trans-

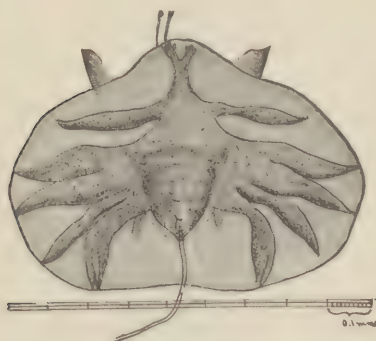


FIG. 46.—Tail, with expanded bursa, of male uncinaria duodenalis. (Tyson.)



FIG. 47.—Anterior end, showing mouth parts, of uncinaria duodenalis, dorsal view. (Tyson.)

versely; tapering to a blunt point anteriorly and with head curved upon the dorsum so as to give a slightly hooked anterior end; on each side of the median line on the ventral side of oral border two hook-like chitinous teeth and on dorsal border on each side of the median line one less curved chitinous tooth; with a dorsal conical tooth extending along back of oral cavity from the base of the cavity; in the oral cavity about the esophageal opening a delicate armature consisting of two dorsal and two ventral lancet-like pieces; posteriorly the body ends in an abruptly pointed tail in a copulatory bursal expansion of the cuticle, this having one dorsal and two lateral lobes; in the folds of the bursa one dorsal subdivided muscular ray, each division ending tridigitally, and on each side symmetrically placed an undivided dorsolateral, a divided lateral, undivided lateroventral, subdivided ventral and undivided small subventral muscular rays; cloacal aperture superterminal; two equal spicules.

The female worm has the general appearance of the male and is shaped anteriorly like it; 12 to 18 mm. long; posteriorly tapering to a finely pointed

¹ Kolle and Wassermann's Handbuch der pathog. Mikroorg., 1913, VIII, 73.

tail; anus subterminal; vulva about the posterior third of the body length; two uterine and ovarian tubes.

The ova are colorless, elliptical, thin-shelled with unsegmented or early segmenting material, 50 to 60 microns long and 30 microns broad.

This worm has a wide distribution both in tropical and subtropical countries. Its habitat is in the duodenum, jejunum, and upper part of the human ileum. Infection with this organism is known as *uncinariasis* or *ancylostomiasis*.¹ A particularly severe type of anemia is set up by this parasite both through the influence of loss of blood and the elaboration of toxic hemolytic material by the parasite. The eggs of this organism are frequently found in the stools and should be carefully searched for in every case of severe anemia. The mode of infection by these organisms may be direct ingestion of dirty water or unclean vegetables, but it is probable that the most frequent method of infection is through the skin, these larvæ attaching themselves to the feet of people walking in infested sand or water.² The larvæ penetrate the skin and make their way through the blood- and lymph-currents to the lungs, whence they penetrate to the air-passages and are supposed to be carried upward toward the mouth by the bronchial mucus and are then swallowed.

(2). *Uncinaria americana*³ (American Hook-worm).

The male parasite differs from the former organisms discussed in being of smaller size (6 to 9 mm. long and more slender than the *duodenalis*), in the smaller size and more conical shape of the head, in having no hooklets on the oral rim, but instead on each side a large ventral and smaller dorsal chitinous lip, extending from the rim toward the median line; in a greater prominence and projection into the oral cavity of the dorsal conical tooth; in the smaller size of the copulatory bursa, its dorsal lobe being subdivided and the ventral margin being extended so as to form an indefinite ventral lobe; and showing the dorsal muscular ray of the bursa divided, each division ending in a bipartite tip.

The female worm differs from the *duodenalis* in being shorter and more slender (8 to 15 mm. long), with similar differences of the anterior end as above outlined for the male; vulva just in front of the middle of body length instead of at the posterior curve as in the *uncinaria duodenalis*.

The ova of this parasite are somewhat larger (68 to 70 microns long and 38 to 40 microns broad) than those of the *uncinaria duodenalis*, but are otherwise similar.

Stiles has found that these parasites⁴ are the common cause of the frequent

¹ See Bruns, Kolle and Wassermann's Handbuch, 1913, VIII, 41; Soltan, Lancet, 1919, II, 690.

² Cort, Augustine and Payne (Jour. Am. Med. Assoc., 1921, LXXVII, 2035) report that hookworm larvæ, under tropical conditions will die out quickly in the soil after the elimination of soil pollution by infected individuals.

³ Also called *Necator americanus*. See Stitt, Jour. Am. Med. Assn., 1912, LIX, 1706; also Stiles, Pub. Health Reports, 1913, XXVIII, 7; also, Ashford, War Dept., Office of Surgeon General, Bull. 2, 1913, 59 and 72; Ferrell, Jour. Am. Med. Assn., 1914, LXII, 1937; Billings and Hickey, Jour. A. M. A., 1916, LXVII, 1908; Frick, Am. Jour. Med. Sc., 1919, CLVII, 189.

⁴ See Glover, Jour. Am. Med. Assn., 1912, LVIII, 1837, for a discussion of *uncinariasis* in oriental immigrants.

"anemia of the South." Smith believes that uncinariasis exists in every case in which "ground-itch" has occurred within eight years and that the disease is rarely ever present in those who have not had this condition during that period. This would point to the general transmission of infection through the skin. The *uncinaria duodenalis* has long been known as the cause of the so-called Egyptian chlorosis, tunnel-workers' anemia, brick-layers' anemia, and other conditions necessitating work in low-lying watery places.

Pseudoparasites.

It not infrequently happens that extraneous substances are found in the feces which very closely simulate a parasite or its ova in appearance. These substances are, for the most part, food residues and should be carefully differentiated by applying tests for cellulose, which will show in all vegetable cells.

Stiebel has described, under the name of *diacanthos polycephalus*, a fragment of the woody portion of a bunch of raisins. Sultzer describes a mulberry seed as a vesicular worm under the name of *ditrachyceros rudis*. Basti-

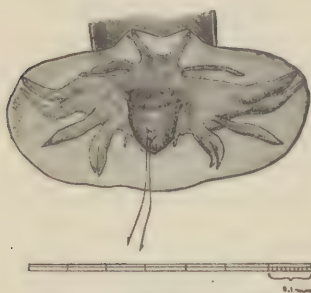


FIG. 48.—Tail, with expanded bursa, of male *uncinaria americana*. (Tyson.)



FIG. 49.—Anterior end of *uncinaria americana*, showing mouth parts (dorsal view). (Tyson.)

ani considers the larynx of a bird which he found in the fecal material as being a biped worm, under the name of *sagitula hominis*. Scopoli regards a fragment of the trachea of a bird as an entozoon form under the name of *physis intestinalis*. A pupil of Moquin-Tandon describes a strip of lettuce under the name of *striatula*, regarding it as a worm intermediate between the ascaris and the oxyuris (Guiart and Grimberty).¹

Perhaps the most frequent pseudoparasite of the feces is the pulp of an orange, it shows in the feces in the form of large oblong masses terminated by two slender extremities, one of which ends in a sort of parenchyma. These vesicular masses are the large cells which secrete the orange juice and which are generally found intact in the fecal material. These have been frequently mistaken for hydatids or for parasitic ova. Certain spores, such as those of the truffle or of lycopodium, have not infrequently been mistaken for parasitic eggs. Moreover, one may find in the fecal material the pollen of the

¹ Francaviglia (Policlinico, 1915, XXII, 1052), reports the presence of blood clots simulating parasites.

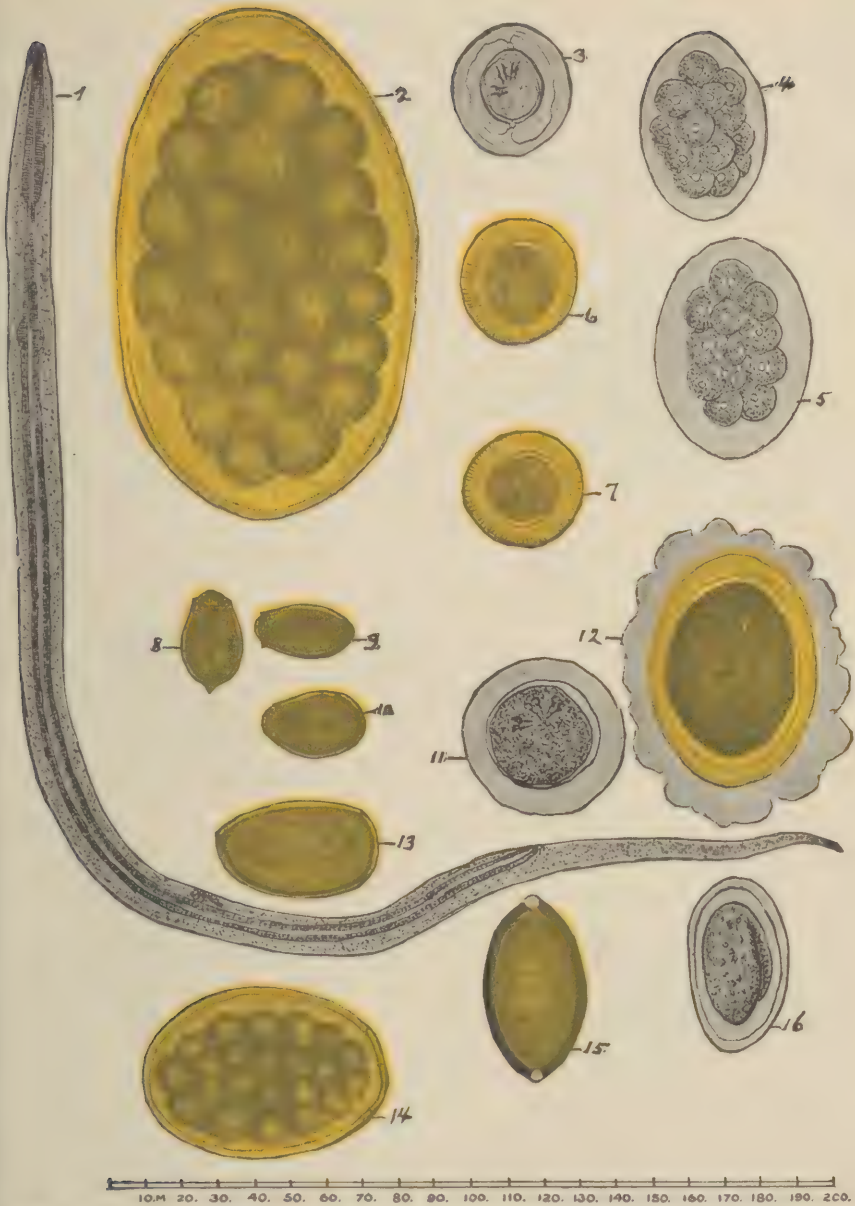


FIG. 50.—Parasitic bodies, ova, and larvæ met in human feces; color approximate only. (Tyson.)

1. Larval strongyloides intestinalis.
2. Ovum of fasciola hepatica.
3. Ovum of tania nana.
4. Ovum of uncinarina duodenalis.
5. Ovum of uncinarina americana.
6. Ovum of tania saginata.
7. Ovum of tania solium.
8. Ovum of opisthorchis sinensis
9. Ovum of opisthorchis felineus.
10. Ovum of cotylogonimus heterophyes.
11. Ovum of tania cucumerina.
12. Ovum of ascaris lumbricoides.
13. Ovum of dicrocoelium lanceatum.
14. Ovum of bothriocephalus latus.
15. Ovum of trichiuris trichiura.
16. Ovum of oxyuris vermicularis.

coniferous plants which very closely simulates parasitic ova. The spines which form the down on certain fruits, such as raspberries, strawberries, peaches, and quinces, so closely resemble parasites that careful study is in some cases essential.

It is, therefore, wise in all cases before pronouncing a finding as one of a parasite to be perfectly sure of your ground.

PLATE VIII.



Katherine Hill.

VEGETABLE CELLS FOUND IN FECES. (AFTER SCHMIDT AND STRASBURGER.)

CHAPTER V

PARASITES

I. GENERAL CONSIDERATIONS

In the previous portions of the work, the writer has introduced those parasites which are more particularly related to the parts under discussion. There are, however, a large number of organisms which do not fall naturally within the scope of any of the chapters outlined for this book. These will, therefore, be discussed in general without much regard to distinct classifications, as many of the subdivisions have been treated previously. This discussion is taken, for the most part, from Tyson.¹

II. TREMATODES (FLUKE-WORMS)

Flukes are naked and unsegmented flat worms, usually of the shape of a leaf or of the tongue (occasionally pyramidal or elongated and more or less cylindrical), provided with incomplete digestive canal (without anus), possessing one or more suckers and occasionally hooklets; with but few exceptions hermaphroditic and, as a rule, presenting a complicated series of metamorphoses in their development.²

In structure it is customary to speak of the surface upon which the genital pore opens as the ventral; this surface commonly shows also the orifices of the mouth and one or more suckers. On the dorsal surface in many occurs the opening of a small canal, spoken of as Laurer's canal, of unknown function. The surface of the body is covered by a fairly thick and firm cuticle, often provided over variable areas with small spines or tubercles. Beneath the cuticle over its internal surface is spread the superficial muscular layer (not showing the structure of muscle of higher animals, however), with longitudinal, circular, and diagonal fibers, while within this along the borders is met the parenchymatous muscle. The general internal tissue of the body, spoken of as the parenchyma, is a fine reticular connective tissue which closely surrounds the various organs.

The suckers of trematodes vary in number and arrangement on the anterior and posterior extremities, the ventral surface and its borders, and in a few cases also on the dorsal surface. Usually the oral opening is surrounded by such a sucker and in addition, in the forms likely to be met with in man, on the ventral surface some distance posterior to the oral sucker is a second, known as the ventral sucker or acetabulum, in the median line. Not infrequently in the lining of these suckers, on their lips, or on the cuticle close to the lips, chitinous hooklets are to be found.

¹ See Ward, *Illinois Med. Jour.*, 1912, XXII, 417; Cort, *Ill. Biological Monographs*, 1915, I, No. 4.

² See Stunkard, *Jour. Parasitol.*, 1916, III, 21.

The alimentary system consists of a mouth, opening in the oral sucker and situated terminally or on the ventral surface of the anterior end of the worm. This cavity continues into a dilated tube with thick walls, the pharynx, this extending posteriorly by a short, straight, and usually narrow esophagus, which divides in the anterior portion of the body into the two intestinal tubes or ceca. At the posterior extremity of the body is a small orifice, the excretory pore, which serves as outlet for a series

of more or less complex canals for the convection of the fluid waste, the arrangement representing a low nephridial apparatus, while the mouth serves as an anus. The reproductive system is highly developed, showing numerous minor variations in the different genera and species.

Adult flukes are parasitic upon a wide range of animal life, including the higher animals, fish, amphibia, reptiles, and birds, living as ectoparasites and endoparasites. The varieties affecting man are comparatively few. The most common parts of the human body to be infested are the intestines, gall-ducts, respiratory tubes, and blood-vessels.

(a) Fasciolidæ.

(1) *Fasciola hepatica*.

SYNONYMS.—*Distomum hepaticum*; *distomum caviæ*; *fasciola humana*, *cladocælium hepaticum*; common liver-fluke.

A comparatively large fluke, measuring 20 to 50 mm. long and 8 to 13 mm. wide, of leaf shape, with anterior extremity prolonged into a small cone; greatest width of body about the anterior third of length; light brown color; cuticle provided with alternating transverse rows of spines, extending on ventral surface to the posterior

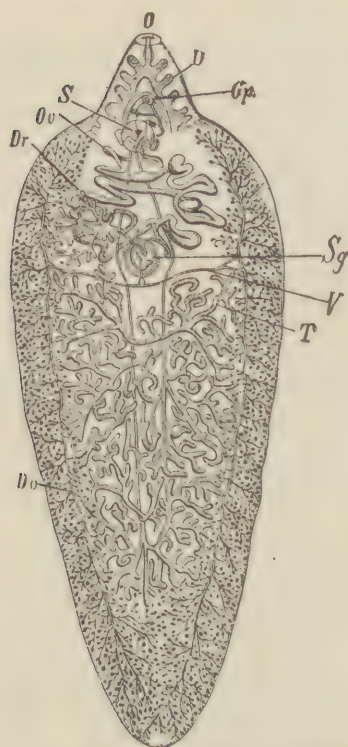


FIG. 51.—Showing the sexual glands of *fasciola hepatica*; 5×1. O, oral sucker; D, intestinal ceca; Do, vitelline glands; Dr, ovary; Ov, uterine canal; T, testes; Sg, "shell gland;" V, transverse vitelline duct; Gp, genital pore; S, ventral sucker. (Tyson after Braun.)

level of testes, but not as far posteriorly on the dorsal surface; the oral sucker at the anterior end of cephalic cone, inclining to ventral surface, 1 mm. in diameter; ventral sucker near anterior end behind cephalic cone, 1.6 mm. in diameter; well developed larynx and short esophagus; intestinal branches extending nearly to the posterior extremity of the worm, approaching the median line posteriorly with few median and numerous lateral branches; excretory pore at posterior extremity, with well-developed system of excretory tubes; genital pore in the median line anterior to the ventral sucker; two large highly branched testes, mostly posterior to the transverse vitelline duct; ovary single, branched, lying in front of testes and to one side

of the median line; the uterus coiled into a rosette, showing as a brown spot just back of the ventral sucker on the ventral surface; vitelline glands numerous, ranging along each lateral border from the level of the ventral sucker to the posterior extremity of the worm; vitelline ducts running transversely at about the end of the anterior third of the body. The ova are yellowish-brown, oval, operculated, and measure 130 to 145 microns in length and 70 to 90 microns in width.

This fluke is a common one in most mammals and has a wide geographical distribution over the world, being found not infrequently in America. Its usual habitat is the gall-ducts, but it has been seen in the gall-bladder, intestines, in the portal and other venous channels and in subcutaneous cysts. The ova appear in the feces and are the chief means of diagnosis of this condition. The embryo which develops from the ovum completes its developmental cycle in the body of a snail, the *Limnaea truncatula*. From the body of the snail the cercariæ escape in the water and become attached to grass or other aquatic material, which is taken in by the animal. Infection in man is quite rare, about 32 cases having been reported in the literature.¹

(2) *Fasciolopsis buski*.

SYNONYMS.—*Distomum buski*; *distomum crassum*.

The length of this organism is variable, ranging between 24 and 70 mm.; breadth 5.5 to 14 mm.; lance-shaped; narrowing more rapidly anteriorly than posteriorly, maximal width about the middle of the length; no cephalic cones; brownish in color; cuticle without spines; oral sucker small and placed on ventral surface of anterior extremity; ventral sucker two or three times as large as the oral, placed near anterior end and showing a saccular distention extending posteriorly; very short esophagus, back of the fairly developed pharynx; the two ceca without branches; genital pore at the anterior quarter of the acetabulum; cirrus pouch large; testicles branched; in posterior part of body one back of the other; uterus in anterior half of body, tortuously coiled; ovary at middle of length of body, to the right of median line; Laurer's canal present; vitelline follicles numerous along lateral margin from the level of the ventral sucker to the posterior extremity; the transverse vitelline ducts at



FIG. 52.—*Fasciolopsis buski*; a, oral sucker; b, acetabulum; c, cirrus pouch; d, vitelline glands; e, "shell gland;" f and g, posterior and anterior testicles; h, ovary; i, cecum; k, uterus. (Tyson after Braun.)

¹ Chandler, Jour. Agric. Res., 1920, XX, 193.

the equator of body. The ova are brownish, ovoid, operculated, and measure 125 microns in length and 75 in width.

Very little is known of the intermediate stage and of the host of Busk's intestinal fluke. This has been found in the small intestine of man and probably arises from eating infected food.¹

(3) *Opisthorchis felineus*.

SYNONYMS.—*Distomum conus*; *distomum lanceolatum* (Siebold); *distomum sibiricum*; *distomum tenuicolle*; the European cat-fluke.

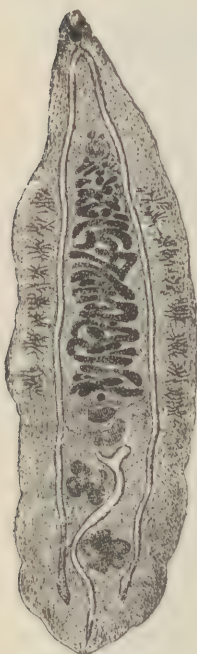


FIG. 53.—*Opisthorchis felineus*; from liver of cat; 10 × 1. (Tyson after Braun.)

Variable in size according to the state of contraction, 8 to 11 mm. long and 1.5 to 2 mm. broad; yellowish-red and nearly transparent; flat and lanceolate; anterior end constricted and attenuated into a cone; posterior end more obtuse; cuticle without spines; oral sucker toward the ventral surface at anterior extremity; ventral sucker at base of cone about one-fourth of body length; pharynx and esophagus of equal length; ceca comparatively straight and unbranched, reaching nearly to the posterior extremity and often seen filled with blood; excretory pore terminal; its tubular vesicle winding in the median line between the testes and branching in front of the anterior testes; testes in the posterior part of body, the anterior four-lobed, the other five-lobed; cirrus and pouch absent; genital pore in the median line in front of the ventral sucker; slightly lobate ovary in the median line anterior to the testes; receptaculum seminis prominent; uterus anterior to the ovary and testes coiled in the middle third

of the body; vitelline follicles occupy about the middle third of the body, beginning anteriorly at the level of the ventral sucker. The ova are oval in shape, operculated, and measure 30 microns in length and 11 in width.

This worm has been found in the gall-ducts of man and other animals, especially in Russia, Siberia, Hungary, and Japan.

(4) *Opisthorchis sinensis*.

SYNONYMS.—*Distomum sinense*; *distomum spathulatum*; *distomum hepatis endemicum seu perniciosum*; *distomum hepatis innocuum*; *distomum japonicum*; *clonorchis sinensis*; the Japanese or Chinese liver-fluke.

This parasite measures 10 to 20 mm. in length and 2 to 5 mm. in breadth; long and lanceolate; reddish and nearly transparent when fresh; cuticle without spines; oral larger than ventral sucker, on the ventral face of anterior extremity; ventral sucker about one-fourth of body length posterior to former; pharynx and esophagus small and a bifurcation of the latter close to oral sucker; ceca unbranched, reaching close to the posterior extremity; excretory system as in the previous species; in the posterior fourth of body the two

¹ See Sweet, Jour. Am. Med. Assoc., 1921, LXXVI, 1819.

testes, one in front of the other, with from four to six dendritic branches; no cirrus or pouch; genital pore in the median line just in front of the acetabulum; ovary tri-lobed and placed just anterior to a large gourd-shaped receptaculum seminis both in the median line and anterior to testes; uterus well-developed and coiled in the middle area of the body between the ovary and ventral sucker; vitelline follicles in the marginal fields along the middle third of the body. The ova are brown, oval, operculated, and measure 27 to 30 microns in length and 15 to 17 in width.

This fluke is comparatively common in Japan and eastern Asia and has been reported in America. It infests the gall-ducts and has been found both in the pancreatic duct and intestine. Little is known of the intermediate hosts.¹

Many other types of these distomata are met with in the liver of various animals and are occasionally found in man. Among these we find the *distomum lanceolatum*, *distomum heterophyes*, *distomum conjunctum*, and *amphistomum hominis* (*gastrodiscus hominis*).

III. Nematodes (Round Worms).

Eustrongylus gigas.

SYNONYMS.—*Ascaris canis et martis*; *ascaris visceralis et renalis*; *strongylus gigas*; *strongylus renalis*; *eustrongylus visceralis*.

The male worm is red in color; 14 to 40 cm. in length and 4 to 6 mm. in thickness; slightly tapering anteriorly; mouth terminal; with a hexagonal orifice surrounded by six lips bearing papillæ; cuticle thin and transparent, finely striated transversely; about 150 papillæ along the longitudinal lines laterally; caudal extremity with an oval plate-like expansion serving as a bursa, its margin bearing small papillæ and slightly indented dorsally and ventrally; single sexual spicule.

The female parasite shows the general appearance and head end as in the male; 20 to 100 cm. in length and 5 to 12 mm. in thickness; caudal extremity obtuse, straight, with anus subterminal; vulva 50 to 70 mm. posterior to mouth; single ovarian and uterine tube plicated from near the anterior end along the intestine nearly to the anus, then returning to the vulva near the anterior end.

The ova are brown, ellipsoid, with thick shell marked by external cribriform depressions, 64 to 68 microns in length and 40 to 44 microns in breadth.

¹ See Gunn, Jour. A. M. A., 1916, LXVII, 1835; Cort, Am. Jour. Hyg., 1921, I, 1.

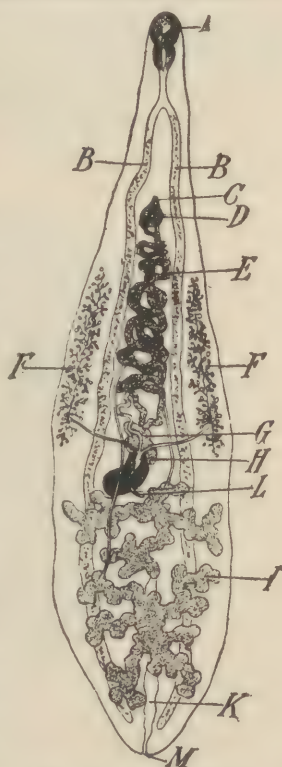


FIG. 54.—*Opisthorchis sinensis*; ventral surface, stretched; a, oral sucker; b, ceca; c, genital pore; d, acetabulum; e, uterus; f, vitelline glands; g, ovary; h, receptaculum seminis; i, Laurer's canal; j, testes; k, excretory canal; m, excretory pore. (Tyson after Braun.)

This worm, which is more common in the dog, has been reported a number of times in man. It is the largest of the nematode worms and has its habitat in the pelvis of the kidney. Little is known of its life history.

IV. PARASITES OF THE SKIN

Arthropoda.

These are bilaterally symmetrical segmented animals whose segments do not correspond, but vary in structure, and which primitively bear upon each segment a pair of jointed appendages. The segments are often more or less fused, thus forming special body-regions which may themselves be more or less fused together as well. The covering of these animals is a com-

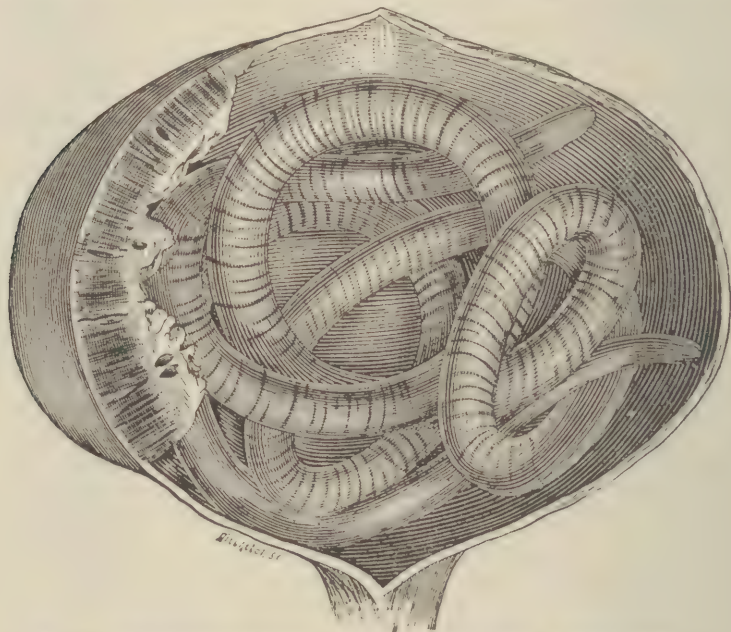


FIG. 55.—*Eustrongylus gigas*: female, natural size, in kidney of dog.
(Tyson after Railliet.)

paratively thick and strong cuticle which remains pliable between the segments of the body and of the jointed appendages, but which commonly becomes hard and shell-like from chitinous or calcareous material directly over the different body-segments and internodes of the jointed appendages. This arrangement requires that in the growth of the individual the firm external covering should from time to time be shed, such changes taking place periodically and being known as *moult*s. While each segment in the primitive animal is provided with a pair of jointed appendages, these in the individual species are often lost from this or that part of the body, or may remain rudimentary and inconspicuous or may take on special features of structure from the assumption of special function which causes their wide departure from the original and common type used for locomotion. The arthropods

commonly reproduce by ovulation, the development of the embryo to the adult often showing more or less complicated metamorphoses. The true parasite forms of the arthropoda thus far met in man are limited to the Arachnoids and Insects.

(A) **Arachnoidea.**

(a) **Sarcoptes or acarus scabiei (the Itch Parasite).**

This parasite is oval in shape, is provided with horns and bristles, is barely visible to the naked eye, the male being from 0.2 to 0.3 mm. in length by 0.145 to 0.19 mm. in breadth; the female is somewhat larger, showing a length of 0.33 to 0.45 mm. and a breadth of 0.25 to 0.35 mm.

The female lies at the end of a burrow in the epidermis, in situations

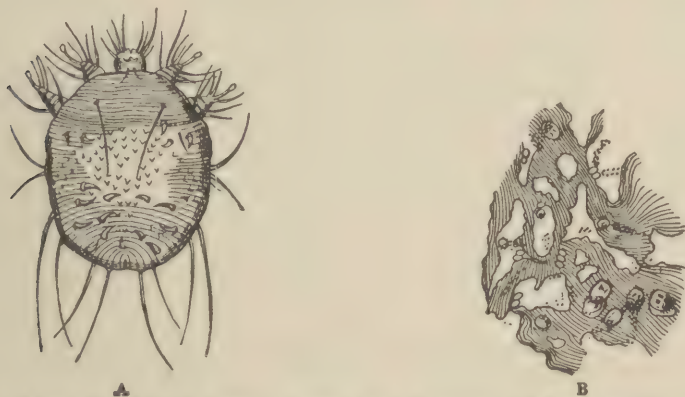


FIG. 56.—*Acarus scabiei*: A, female, dorsal view; B, portion of human epidermis, showing burrows with contained ova and young acarians. (Gould.)

where the skin is most delicate, as between the fingers, at the elbows, under the knees, and in the groin. In this burrow, which varies from a few millimeters to a centimeter in length, the female deposits her eggs, after which she dies. The eggs hatch in from 4 to 8 days, and in about 14 days the larvæ are sufficiently matured to make their own burrows.

The disease is communicated either by the clothing or by personal contact. To demonstrate the parasite, the burrow is opened with a needle and the female pressed out on a slide, which is then covered and examined.

(b) **Demodex folliculorum.**

This parasite is very small, varying in length from 0.3 to 0.4 mm. It is somewhat cylindrical, tapering to an obtuse point at the posterior end. This parasite has its habitat in the sebaceous follicles, especially of the face and nose.

(c) **Leptus autumnalis (Harvest-bug).**

This parasite is also known as *trombidium irritans* or red jigger. This is a minute red parasite, from 0.3 to 0.5 mm. long, which has three pairs of legs, with rows of bristles upon its back and belly. It prevails in summer on

grass and plants and attaches itself to the skin of man by its hooklets. The condition of the skin due to the infection with this parasite is known as trombidiosis.¹

(B) Insecta.

(a) Hemiptera.

(1) *Pediculus capitis* (Head-louse).



FIG. 57.—*Demodex folliculorum*: from dog, enlarged. (Tyson after Braun.)

The male is from 1 to 1.5 mm. long, the female 1.8 to 2 mm. long. The color of the parasite varies somewhat with the race of its host. In the Caucasian it is gray with a dark border, in the Negro and Chinamen it is much darker in color. The eggs are 0.6 mm. in length and are attached to the hairs, forming the so-called "nits." These nits are whitish oval masses which are easily visible.

This parasite, while usually found upon the hair of the head, may be found in other portions of the body.² The symptoms may be severe or very slight.

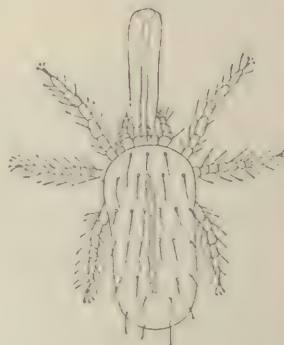


FIG. 58.—*Leptus autumnalis*; enlarged. (Tyson after Braun.)

(2) *Pediculus vestimenti* (Body-louse).

This parasite is considerably larger than the former, being from 2 to 5 mm. long and whitish-gray in color, the back part of the body being wider than the thorax. The antennæ are longer than are those of the head-louse. The eggs are from 0.7 to 0.9 mm. in length, about 70 being laid by each female.

This parasite is found upon the clothing in which it deposits its eggs, especially about the neck, back and abdomen.

(3) *Pediculus pubis* (phthirius inguinalis or Crab-louse).

This parasite is smaller than the head-louse, grayish-yellow or grayish-white in color, the male being from 0.8 to 1 mm. in length, the female about 1.12 mm. in length. The eggs are pear-shaped, from 0.8 to 0.9 mm. in length and from 0.4 to 0.5 mm. in breadth.

This parasite infests the parts of the body covered by the shorter hairs, such as the pubis, axilla, eye-brows, and chest.

¹ See Olson, Jour. Am. Med. Assn., 1915, LXIV, 2060; Nagayo, Miyagawa, Mitamura and Imamura, Jour. Exper. Med., 1917, XXV, 273.

² Goldberger and Anderson (Pub. Health Rep., 1912, XXVII, 297) show that typhus fever may be transmitted by this parasite as, also, by the body louse. See, also, Nicolle, Comte and Conseil, C. R. Acad. Sc., 1909, CXLIX, 486; Ricketts and Wilder, Jour. Am. Med. Assn., 1910, LIV, 1304; Goldberger and Anderson, Pub. Health Rep., 1910, XXV, 177; Ibid., 1912, XXVII, 835; and Jour. Am. Med. Assn., 1912, LIX, 514.

(4) *Cimex lectularius* (*acanthia lectularia* or Bed-bug).

While, strictly speaking, the bed-bug is not a parasite of man, yet as its habitat is the bed, bedding, and walls of the sleeping apartment of man, it may be considered as indirectly parasitic. It usually emerges at night from

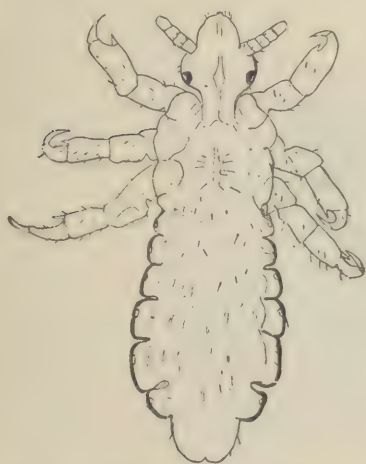


FIG. 59.—*Pediculus capitis*, $\times 15$.
(Tyson after Braun.)

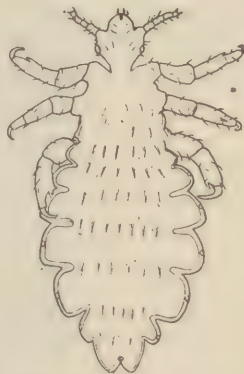


FIG. 60.—*Pediculus vestimenti*, $\times 10$. (Tyson after Braun.)

its lodging for the purpose of securing its nourishment in the blood of its victims.

This parasite is reddish-brown in color, oval in shape, from 4 to 5 mm. in length and 3 mm. in breadth. These insects, if crushed between slides

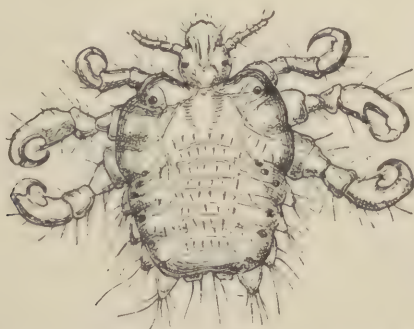


FIG. 61.—*Pediculus pubis*. (Tyson after Braun.)

or as more usual between the hand and a part of the victim's body, have a characteristic odor very much resembling kerosene. The blood is drawn from the victim by means of a long proboscis. The eggs are approximately 1.12 mm. in length and require about 11 months for their development to the sexually ripe insect. These eggs are retained in the crevices of the bed, floors,

furniture, wall-paper, and other parts of the dwelling so that the complete removal of these eggs and parasites is a matter of some difficulty.¹

That these insects have more or less importance from the standpoint of transmission of disease from one person to another must be remembered. Individuals vary in their susceptibility to the bite of the bed-bug, some being indifferent to it while others are markedly affected by it.



FIG. 62.—*Pulex irritans*, $\times 14$. (Tyson after Braun.)

(b) **Diptera.**

(1) ***Pulex irritans* (Common Flea).**

The male is from 2 to 2.5 mm. in length, the female as much as 4 mm. It is a red or brownish-red insect, having a laterally compressed body, an oral haustellum, serrated soft mandibles, a tongue sheathed in an inferior labium, and a pair of labial four-jointed palpi. Each of the triple segments of the thorax bears a pair of five-jointed double-clawed legs. The female deposits her eggs, not on the human being, fortunately, but in the fissures, crevices, or holes of garments or furniture which may be accessible.

(2) ***Pulex penetrans* (Sand-flea or Jigger).**

This parasite is a minute, brownish-red, egg-shaped insect which penetrates the skin of man. The female is the infecting insect and produces painful irritation and even suppuration.

Vegetable Parasites.

(1) ***Achorion Schönleinii*.**

This organism is the cause of the disease known as *favus* or *trinea favosa*. This fungus invades the root sheaths, the bulbs, and the shafts of the hair filaments of the scalp, but it also occurs upon the "non-hairy" portions of the skin and upon the nails.² The spores gain access to the deeper layers of the skin and develop around the hair-shaft, forming a characteristic yellowish cup-shaped crust which has a peculiar mouse-like odor.

¹ See Rucker, Pub. Health Reports, 1912, XXVII, 1854; Thomson, Ann. Trop. Med. and Parasitol., 1914, VIII, 19.

² See Foster, Jour. Am. Med. Assn., 1914, LXIII, 640.

In searching for this parasite, a favus crust is softened by the addition of a few drops of water or dilute sodium hydrate solution and placed upon a slide and examined with the high-power dry lens. The hairs may also be examined in the same manner or may be stained by methods outlined in the discussion on *Tinea trichophytina*.

The mycelial threads appear as narrow, flattened, ramifying, short or elongated, linear cells or tubes, which may be simple and empty, or be divided more or less regularly by transverse partition walls transforming the longer and simple into shorter and compound cells. The latter often contain in their cavities sporules clinging to either side, in which case the mycelial threads are termed sporophores. The conidia are encapsulated or are strung together



FIG. 63.—*Pulex penetrans*: young female, enlarged. (Tyson after Braun.)

like the beads of a necklace, and appear as round, oval, angular, or very irregularly contoured bodies. These mycelial threads branch at right angles, the spores measure from 3 to 10 microns in diameter (Hyde).

(2) *Trichophyton megalosporon endothrix*.

This organism is the cause of *tinea circinata* (herpes tonsurans, ringworm of the body), and of *tinea sycosis* (hyphogenous sycosis, *tinea barbæ*, ringworm of the beard, barber's itch).

The trichophyton is composed of spores which vary greatly in size, but which, as a rule, are somewhat larger than those of the type next to be discussed.¹ They are frequently cuboidal, oval, or irregularly rounded, but their chief characteristic lies in their arrangement in lines or chains, extending up and down the hair shaft. The mycelium is found without, but never within the hairs (Hyde).

These fungi may be stained by the method of Morris and Calhoun. The hair is first washed in ether to remove all fatty debris; it is then put for one

¹ Priestley (Ann. Trop. Med. and Parasitol., 1914, VIII, 113) reports the presence of the *microsporon scorleum* in a case of ringworm. See Ormsby and Mitchell, Jour. A. M. A., 1916, LXVII, 711; Muijs, Nederl. Tijdschr. v. Geneesk., 1916, II, 1985.

or two minutes in Gram's iodine solution and is stained after drying for from one to five minutes in gentian-violet. It is again dried and treated for a minute or two with the iodine solution and for an equal length of time in aniline oil containing pure iodine, after which it is cleared with aniline oil, washed in xylol, and mounted in Canada balsam.

(3) **Microsporon audouini (Trichophyton Microsporon).**

This parasite appears under the microscope chiefly in the form of a large number of round spores, irregularly grouped or massed about the follicular portions of the hair. Mycelial threads, large and branching, are often seen

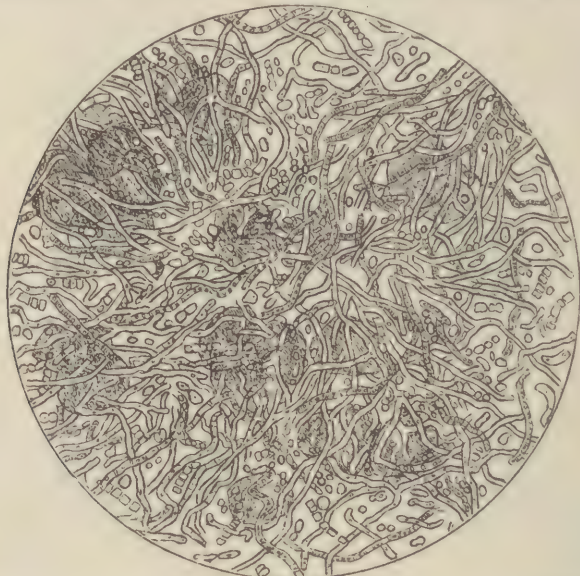


FIG. 64.—*Achorion schönleinii*, $\times 500$ diameters. (Van Harlingen.)

within the hair. The sheath of spores surrounding the hair is often continued upward for $\frac{1}{16}$ to $\frac{1}{8}$ inch above its exit from the follicle and may be recognized as a whitish or grayish coating of the hair. These mycelial threads are all within the hair proper, thus differing from those of the trichophyton which are never within the hair; after repeatedly dividing and subdividing they terminate on the outer surface of the shaft in fine filaments, at the extremities of which are the spores. This parasite is the cause of the disease *tinea tonsurans*, or ringworm of the scalp.¹

(4) **Microsporon furfur.**

This parasite is readily recognized by the microscopic examination of the scales scraped from the skin. Innumerable clustered spores, highly refractive and resembling in their circular and oval contours droplets of oil, are quite characteristic. The mycelial threads are not usually branched, but lie in a close network, among which sporophores are distinguishable, with conidia and terminal elements emerging at one extremity of the spore case. Both

¹ See Beeson, Jour. Cut. Dis., 1915, XXXIII, 731.



FIG. 65.—Normal hair, $\times 900$.



FIG. 66.—Hair showing trichophyton endo-ectothryx, $\times 900$.

elements of this organism are more readily stained by the aniline dyes than are those of the trichophyton or favus. This organism is the cause of the condition known as *tinea versicolor*.

(5) **Microsporon minutissimum.**

This organism is the etiologic factor of *erythrasma*. It is characterized by the extreme delicacy and fineness of its threads and very minute spores. The threads are either simple, cylindrical bodies of variable size or they may exhibit partition septa, may divide dichotomously, and may terminate in hooked or knobbed expansions. The largest transverse diameter is 0.6 micron; in length the mycelium presents the greatest variations.



FIG. 67.—Hair showing *microsporon audouinii*, $\times 900$.

(6) **Blastomycetes.**

These organisms may be found in the cutaneous eruptions of the skin in *blastomycosis* and may be described as follows, according to Montgomery and Ormsby. In unstained preparation the organisms appear as round or oval bodies with a double-contoured highly refractive capsule. Within the capsule, in many instances, granules or spore-like bodies can be distinguished. The addition of a 1 to 10 per cent. solution of potassium hydrate to the specimen under examination facilitates the recognition of these bodies. In stained sections the double contoured, homogeneous capsule is usually separated from a finely or coarsely granular protoplasm by a clear space of varying width. Vacuoles of different sizes are found in some organisms. In both pus and tissue, organisms in pairs or in various stages of budding are commonly seen.¹ The parasite, as a rule, varies in size from 7 to 20 microns, though slightly smaller and much larger forms occur in some cases.

¹ See Whitman, Jour. Infect. Dis., 1913, XIII, 85. See a long series of articles by various authors in Arch. Int. Med., 1914, XIII, 509 ff.; Wade, New Orleans Med. and Surg. Jour., 1915, LXVIII, 287; Wade and Bel, Arch. Int. Med., 1916, XVIII, 103; Watts and Wells, Tr. Chicago Path. Soc., 1916, X, 92.

The organisms are readily obtained in pure culture from unbroken abscesses, from miliary abscesses in the borders of the cutaneous lesions, and from the miliary nodules and abscesses in the deep-seated organs. The peculiarities of the cultures of blastomycetes must be looked for in other works. Microscopically, the organism obtained in culture appears at first as a fine, branching mycelium with a few small spore-like bodies. Later a

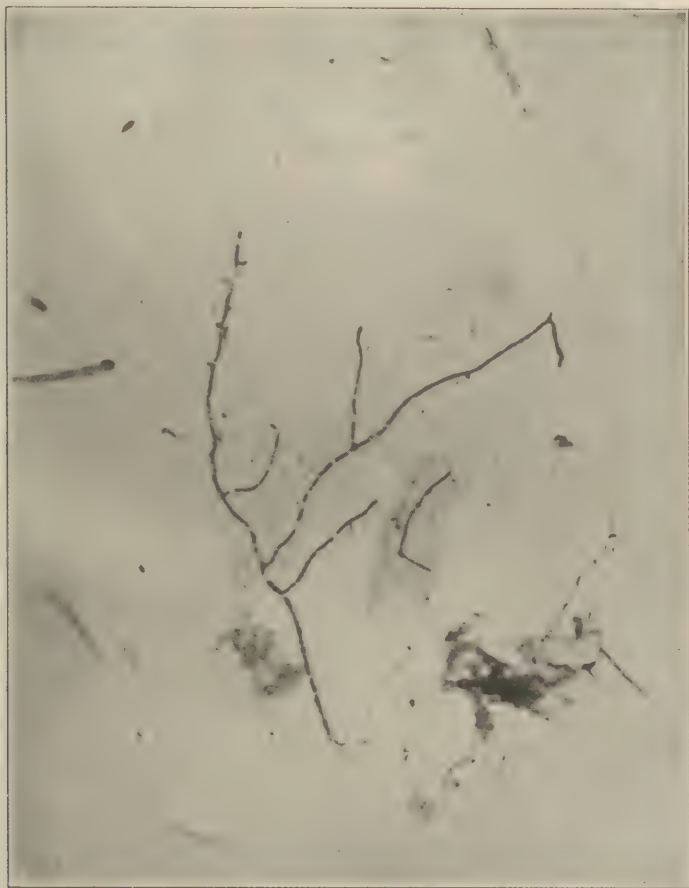


FIG. 68.—Mycelial threads of blastomycetes from old agar culture.
(From photograph by W. A. Pusey.)

large, segmented, often pod-like mycelium appears, together with large, round, or oval bodies with bud-like projections.

The condition known as *coccidioidal granuloma*, which is especially observed in California, has often been confused in the literature with blastomycosis. It is, however, now well established that the etiologic factor of this disease is the *coccidioides immitis*, an organism somewhat similar to but differing distinctly in many respects from the blastomycetes. This organism

was first reported by Posadas and Wernicke, but was later classified by Rixford and Gilchrist and cultivated by Ophüls.¹

(7) *Sporothrix Schenckii*.

This organism is the cause of sporotrichosis and was first recognized by Schenck, Hektoen and Perkins.² Since its discovery a large number of cases have been identified in all parts of the world, especially in the middle-western portions of the United States. The lesions in this condition resemble, at times, those of tuberculosis, while, occasionally, they appear more like those of syphilis. The chief characteristics are the sharply defined, painless, cutaneous or subcutaneous abscesses which follow the lymphatics and do not yield to the ordinary surgical procedures. In living tissues the sporothrix organisms appear as elongated or oval bodies fairly uniform in

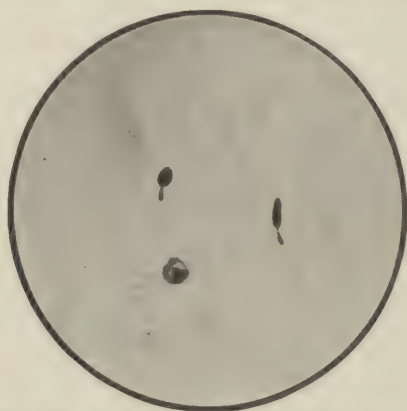


FIG. 69.—Budding forms of *Sporothrix Schenckii* in pus, $\times 1200$. Gram's Stain. (Courtesy of Dr. D. J. Davis.)

size and often showing distinct budding processes. The arrangement is usually single, but frequently two or more are found end to end or arranged radially about a central spore. Branched mycelial filaments are not found in the living tissues but they develop abundantly on artificial media, forming, at their sides and extremities, numerous spores, oval in shape and differing decidedly in appearance from the typical tissue forms. These latter forms may be found, however, in small numbers in artificial cultures (Davis). Smears from the lesions are made in the usual way. To obtain cultures proceed as follows: Some of the light yellow pus is taken, by aseptic methods, from the suspected lesion, is transferred either to glucose or maltose agar, or blood agar, and is kept at room temperature. After a few days small

¹ See MacNeal and Taylor, *Jour. Med. Research*, 1914, XXX, 261; Cooke, *Arch. Int. Med.*, 1915, XV, 479; Brown and Cummings, *Ibid.*, 608; Dickson, *Arch. Int. Med.*, 1915, XVI, 1028; Cummins and Sanders, *Jour. Med. Research*, 1916, XXXV, 243; Lipsitz, Lawson and Fessenden, *Jour. A. M. A.*, 1916, LXVI, 1365; Lipsitz, *Jour. Missouri State Med. Assoc.*, 1916, XIII, 534; Pierson, *Jour. A. M. A.*, 1917, LXIX, 2179, reports a case of torula infection in man; Helsley, *Ibid.*, 1919, LXIII, 1607; Pedroso, *Ann. Paulistas de Med. e Cir.*, 1919, X, 103; Bowman, *Amer. Jour. Roentgenol.*, 1919, VI, 547; Lynch, *Southern Med. Jour.*, 1920, XIII, 246; Haughwout, *Jour. Am. Med. Assoc.*, 1921, LXXVII, 940.

² See Gougerot, in Kolle and Wassermann's *Handb. d. path. Mikroorg.*, 1912, V, 211.

points appear from which circular growths with marked striations develop in 10 to 12 days. Later these may show a deep black pigmentation. Smears made from these cultures show a typical sporothrix, the mycelium being coarse ($2\ \mu$ broad) and forming a dense, branching, septate network. Spores (4 to $5\ \mu$ long and 2 to $3\ \mu$ broad) develop by budding either from lateral or terminal filaments or from the sides of the thread. The organism stains readily with the ordinary dyes and is, also, Gram-positive.¹

(8) Negri Bodies.

Considerable discussion has centered about these bodies as the etiologic factor of rabies (hydrophobia), but now, thanks to the successful experiments of Noguchi² in cultivating them, the subject must be regarded as practically settled in the affirmative. These bodies are found in almost all of the nerve cells of the central nervous system but are more numerous and more easily found in the cells of Ammon's horn, cortical tissue in region of the fissure of Rolando, and in the Purkinje cells of the cerebellum. They are also present in the salivary glands³ of the infected animal but are not readily detected therein. Their demonstration in the cells of the brain is regarded as equivalent to a positive diagnosis of rabies. As they have never been found in any condition other than hydrophobia, they must be regarded as specific.

These bodies were originally described by Negri,⁴ although Galtier⁵ and Pasteur⁶ had previously demonstrated the infectious character of rabies. Since that time many workers have confirmed their findings and have added much to our knowledge of these bodies.⁷

They are found lying in the cytoplasm of the nerve cells or in their branches, occasionally outside, and appear as round, oval, triangular, pear-shaped, spindle- or sausage-shaped bodies. They vary in size from $0.5\ \mu$ to $20\ \mu$ in diameter, the size increasing with the stage of the disease. They show a hyaline cytoplasm with an entire margin and with one or more inner bodies (nuclei) having a more or less complicated and regular structure. There do not appear to be any vacuoles in the smear preparations but in the

¹ See Hamburger, Jour. Am. Med. Assn., 1912, LIX, 1590; Sutton, Ibid., 1913, LX, 115; Chipman, Jour. Cutan. Dis., 1912, XXX, 339; Adams, Journal Lancet, 1912, XXXII, 395; Jour. Am. Med. Assn., 1913, LX, 1784; Taylor, Ibid., 1913, LX, 1142; Davis, Jour. Infect. Dis., 1913, XII, 453; Hamburger, Illinois Med. Jour., 1914, XXV, 99; Dominguez, Med Record, 1914, LXXXV, 608; Davis, Jour. Infect. Dis., 1914, XV, 483; Sutton, Jour. Am. Med. Assn., 1914, LXIII, 1153; Meyer and Aird, Jour. Infect. Dis., 1915, XVI, 399; Davis, Ibid., 1915, XVII, 174; Campana, Riforma Med., 1915, XXX, 925; Bolognesi, Policlinico, 1916, XXIII, 129; Burovi, Russ. Vrach, 1916, XV, 876; Accame, Semana Med., 1916, XXIII, 680; Spoor, Jour. A. M. A., 1917, LXVIII, 1458; Moore and Davis, Jour. Infect. Dis., 1918, XXIII, 252; Lane, Oklahoma State Med. Assoc. Jour., 1920, XIII, 41; LeBlanc, Ill. Med. Jour., 1920, XXXVIII, 516; Wohl, Nebraska State Med. Jour., 1920, V, 355; Beatty, Dublin Jour. Med. Sc., 1921, IV, 116; Tennant and Dennis, Colorado Med., 1921, XVIII, 165; Nellans, Jour. Am. Med. Assoc., 1922, LXXVIII, 802.

² Jour. Exper. Med., 1913, XVIII, 314. See, also, Levaditi, C. R. Soc. de Biol., 1913, LXXV, 505; Kraus and Barabara, Deutsch. med. Wchnschr., 1914, XL, 1507; Noguchi, Berl. klin. Wchnschr., 1914, XLI, 1931; Proescher, New York Med. Jour., 1914, C, 953.

³ See Jackson, Jour. Inf. Dis., 1921, XXIX, 291.

⁴ Ztschr. f. Hyg., 1903, XLIII, 507; XLIV, 520; 1909, LXIII, 421.

⁵ C. R. Acad. d. sc., 1879, LXXXIX, 444.

⁶ Ibid., 1881, XCII, 159.

⁷ See Babes, Traite de la rage, Paris, 1912; Proescher, Berl. klin. Wchnschr., 1913, L, 633; Poor and Steinhart, Jour. Infect. Dis., 1913, XII, 202; Luzzani, Ann. de l'Inst. Pasteur, 1913, XXVII, 907 and 1039; LeComte, Jour. Am. Med. Assn., 1914, LXIII, 1658.

tissue specimens vacuoles are quite distinct (probably artefacts). Most of the bodies show chromatoid granules of varying size and number and arranged about the nucleus like a ring. In stained specimens these granules are seen as basophilic granules, rods or circles. They are, therefore, properly classified as protozoan parasites.

To demonstrate these Negri bodies in the brain cells of a suspected animal, the following method of Williams and Lowden¹ is recommended for diagnostic purposes. A fragment of the gray substance from the cerebral cortex in the region of the fissure of Rolando, another from Ammon's horn and a third from the cerebellum, is taken from a section made at right angles to the surface and placed on slides about 1 inch from their ends. A cover-glass is now pressed upon it until it is spread out in a moderately thin layer; the cover-glass is moved slowly and evenly over the slide, leaving the first portion of the slide clean. Use slight pressure in making the smear, pressing rather more on the edge of the cover-glass away from the end of the slide toward which the cover-glass is moving, thus driving more of the nerve tissue along the smear and producing more well-spread nerve cells. Dry in air and stain as follows.

Fix the air-dried smears in methyl alcohol for five minutes. Pour on the slide the staining solution and allow to stand for one-half to three hours. The staining solution is made by adding 10 drops of Giemsa's stain to 10 c.c. of distilled water made alkaline by the previous addition of 1 drop of 1 per cent. potassium carbonate solution. The longer period of staining is preferable, as overstaining does not occur even after 24 hours. Pour off the stain, wash in running tap-water for one to three minutes and dry with filter paper. If the smear be thick, dip it in 50 per cent. methyl alcohol before it is washed in water.

The cytoplasm of the Negri bodies stains blue, while the central bodies and chromatid granules stain a blue-red or azur. The larger bodies have usually a somewhat darker blue tint than the smaller ones. The cytoplasm of the nerve cells stains blue, while the nuclei are red, the nucleoli are a dull blue and the red cells a pink-yellow.

If it is desired to make a quick examination proceed as follows, although this method is not to be advised. Fix in methyl alcohol for five minutes and add equal parts of Giemsa's stain and distilled water for ten minutes. Wash, dry and examine.

Mallory² recommends the following technique. The smears made as above described are fixed in Zenker's solution for one-half hour; after being rinsed in tap-water they are placed successively in 95 per cent. alcohol, containing sufficient iodine to make a port-wine color, one-fourth hour, 95 per cent. alcohol one-half hour, absolute alcohol one-half hour, 10 per cent. aqueous solution of eosin for 20 minutes, rinse in tap-water, place in alkaline methylene-blue solution for 15 minutes, differentiate in 95 per cent. alcohol from one to five minutes, dry with filter paper and examine. The cytoplasm of the Negri bodies is a magenta, the central bodies and chromatoid granules are a very dark blue, the nerve cell cytoplasm a light blue, the nucleus a darker blue and the red cells a brilliant pink.

¹ Jour. Infect. Dis., 1906, III, 452.

² Pathological Technique, Philadelphia, 1911.

Van Gieson¹ recommends the following: To 10 c.c. of distilled water add 3 drops of a saturated alcoholic solution of rose-aniline violet and 6 drops of Loeffler's methylene-blue solution. Fix the smears in methyl alcohol, pour the above stain on them and warm until steam rises. Pour off the stain, rinse in water, and allow to dry. The stain must be prepared fresh each time as it is not permanent. The cytoplasm of the bodies is a deep and distinctive red, their inner structures are a dark blue, the nerve cell light blue and the blood cells a pale salmon red.²

Noguchi³ has succeeded in cultivating these bodies from infected animals and has, in turn, reproduced rabies by inoculating cultures containing the granular, pleomorphic, or nucleated bodies. He has, then, recovered the granular and nucleated bodies from the brain of the inoculated animals. The specificity of these bodies would seem, therefore, to have been definitely established. See, however, Moon⁴ and Williams.⁵

¹Centralbl. f. Bakteriol., I. Abt. 1907, XLIII, 205

²See Kotsevaloff, Russky Vrach., 1914, XIII, 616.

³Jour. Exper. Med., 1913, XVIII, 314.

⁴Jour. Infect. Dis., 1913, XIII, 232.

⁵Jour. Am. Med. Assn., 1913, LXI, 1509.

CHAPTER VI

THE URINE

I. GENERAL CONSIDERATIONS

The examination of the urine is one of the most important features of clinical diagnosis. So constant are the physical and chemical properties of the normal urine that any marked abnormality is easily detected. The relation between the kidneys and the blood is so close that the kidneys soon excrete any abnormal substances which have found their way into the blood-current. For this reason we find in the urine the abnormal products of perverted metabolism of the system or of special organs. It is true that the urinary findings may be, in any special case, secondary to those of the blood or of clinical examination, but in such conditions we may detect substances in the urine, which put us on our guard against making a specific diagnosis or point out the way to a correct differentiation. More or less marked changes in the character of the urine will occur whenever a pathologic condition exists anywhere in the system. These changes may not always be sufficient to be of direct diagnostic value, but in many cases may settle a differential diagnosis.¹

In the urine we find excreted the products arising from the metabolism of the various proximate principles, both of the tissues and the food. Knowing the intake of such material, we are able from our examination of the urine to judge of the manner in which the system is handling the material brought to it. In recent years the study of the metabolism in various conditions has been so extended that determinations which a few years ago were unusual are now matters of almost daily routine. An examination of the urine will frequently reveal the presence of irregular digestive and absorptive powers of the intestines through the appearance of certain abnormal products of protein decomposition. Moreover, the study of the nitrogen partition of the urine is taking on increasing importance from day to day, so that the estimation of the factors determining this division should be possible by any one attempting to follow the metabolic activity of the system in any specified condition.

When the oxidative powers of the system are lessened, we find the urine showing abnormal products as an indication of such deficiency. These products are more or less characteristic and may be determined with a great degree of exactitude. Thanks to the work upon metabolism in diabetes, for instance, we now know that the glycosuria is clinically not of as great importance as is the presence of many other abnormal products associated with the sugar; in other words, a glycosuria must not be considered as identical with diabetes.

The system has a definite disintoxicating power toward certain noxious

¹ See Freeman, *Jour. Am. Med. Assn.*, 1914, LXIII, 1802; Diner, *New York Med. Jour.*, 1915, CI, 1007; Kilduffe, *Arch. Diag.*, 1915, VIII, 383; Stark, *Jour. Lab. and Clin. Med.*, 1916, II, 134.

substances, whether introduced from without or formed within. While the blood is of special importance in such processes as far as bacterial products are concerned, an examination of the urine will frequently reveal much information regarding the metabolic toxins or the medicinal poisons. The estimation of the conjugated glycuronic and sulphuric acids of the urine will throw much light on the degree of this activity. In this connection it may be mentioned that indican, a product of bacterial decomposition of protein in the intestinal canal, may be taken as a direct indicator of the degree of such decomposition, but that we must not assume that all of the conjugated acids have such an origin.

Besides these general indirect points of interest, an examination of the urine will often reveal a direct anatomical lesion of the kidneys. Time was when we regarded the mere presence of albumin in the urine as indicative of a kidney lesion, but we know that a thorough clinical examination is necessary before a diagnosis is possible. Albumin may or may not mean kidney trouble and may even be purely physiologic. Too much stress can hardly be laid upon the necessity of closely associating the urinary findings in any condition with the clinical symptoms of the case. The writer will have much to say later regarding the various abnormalities of the urine, but he wishes to impress at this point the fact that no finding, no matter how abnormal it may seem, should be considered absolutely pathognomonic, without taking into consideration the clinical manifestations of the case. An albuminuria, glycosuria, cylindruria, or pyuria may mean one thing at one time and another at a second period, so that the worker is cautioned against jumping at conclusions. The urine will yield much information, providing the worker knows how to interpret his findings. A laboratory worker must be cautious in his attitude and report his findings without any attempt at interpretation, unless he is aware of the clinical history of the case in point.

The writer must refer to works on physiology for the various theories which have arisen from time to time regarding the mechanism of secretion of the urine. Suffice it to say at this point that the urine is excreted through the activities of the kidneys, the water and salts being secreted by the glomeruli, while the majority of the excretory products are eliminated by the vital or selective activity of the epithelium of the renal tubules. It is evident, therefore, that the physical and chemical characteristics of the urine will depend both upon the blood-pressure¹ within the capillaries and the rate of flow through these vessels as well as upon the condition of the secreting epithelium.

Collection and Preservation of the Urine.

In all urinary examinations in which quantitative relations are to be

¹ See Lawrence (Am. Jour. Med. Sc., 1912, CXLIV, 330) who shows that the amount of blood in a unit of time is of much more importance than the pressure. See, also, Leschke, *Ztschr. f. klin. Med.*, 1914, LXXXI, 14; Folin and Denis, *Jour. Biol. Chem.*, 1915, XXII, 321; Davis, *Jour. Urol.*, 1917, I, 113. Richards (Am. Jour. Med. Sc., 1922, CLXIII, 1) has shown that, even under conditions in which the blood flow and blood volume are not materially changed, variations in the flow of urine follow blood pressure changes in the kidney and that the glomerulus seems to filter the urine rather than secrete it. He further shows that nervous stimuli and chemical substances may exert different degrees of effective influence on the afferent and efferent vessels of the glomerulus and that this may be a factor in the automatic regulatory control of glomerular filtration. In this connection see Yoshimura, *Tohoku Jour. Exp. Med.*, 1920, I, 113; Richards and Plant, *Amer. Jour. Physiol.*, 1922, LIX, 144.

studied it is necessary that a portion of the total 24-hour specimen be examined. This should be thoroughly mixed and carefully measured. The composition of the different voidings is so variable that no definite idea regarding the elimination can be gained from a single specimen.

If a mere qualitative examination is to be made, a single specimen may be studied. In chronic nephritis, for instance, the morning urine may show points of interest as compared with that voided in the evening after a day's activity. In diabetes it may be desired to study the effect of a carbohydrate meal upon the sugar excretion. This may best be done by an examination of the urine passed three or four hours after such a meal. The microscopic examination is best made as soon as possible after voiding, but it is to be remembered that much variation may be noted in the sediment of the different voidings.

In making the 24-hour collection, the patient is instructed to empty his bladder at a specified time, preferably at 7 A. M. This portion is thrown away and all urine passed from that time until the bladder is emptied at 7 A. M. the next day is saved. If one desires to separate the day and night urine, the voidings from 7 A. M. to 7 P. M. may be kept in one container and those from 7 P. M. to 7 A. M. in a second vessel properly labeled.

A thoroughly clean bottle of one-half to one gallon in capacity should be used as the container.¹ This should be well corked after each addition of urine and kept in a cool place. As urine undergoes decomposition more or less readily, depending upon bacterial activity, some preservative should be added to prevent such processes. The writer is accustomed to advise the use of a slight excess of chloroform. This may be removed by heating the urine and will not then interfere with the later reactions. If not removed, its presence will lead to a pseudocarbohydrate reaction. Three or four drops of formalin may be added for each pint of urine. This is an efficient agent, but it will lead to reactions simulating those for sugar and even albumin, and, moreover, will introduce a crystalline compound of formalin and urea into the sediment as well as markedly interfering with the bile, urobilin² and indican tests. Thymol may be added, but this may give a reaction similar to those for bile pigments, indican and albumin. Camphor,³ chloral, and boracic acid have been used, but do not possess any virtues over the other preservatives mentioned. In any case, the worker should be on his guard in reporting abnormal findings without convincing himself that the reaction is not due to an added preservative.

II. PHYSICAL PROPERTIES

(1) Quantity.

The amount of urine passed within 24 hours depends upon several factors and varies both for individuals and for different races of people. It is self-evident that under normal conditions, the amount of urine will vary with the

¹ See Folin and Denis, *Arch. Int. Med.*, 1915, XVI, 195.

² See Hausmann, *Deutsch. med. Wchnschr.*, 1913, XXXIX, 1685; Schmitz, *Ibid.*, 1914, XL, 128.

³ See Rosenbloom, *New York Med. Jour.*, 1914, XCIX, 735.

quality and quantity of the substances to be excreted, the condition of the renal parenchyma, the pressure and rate of flow of the blood-current, the vasomotor disturbances, the stage of digestion, the loss of fluid in the perspiration as influenced by the surrounding temperature, amount of exercise, and extent of fluid intake; upon the weight of the subject, the sex, and age.¹

As a rule, the quantity of urine excreted varies between 1,200 and 1,500 c.c. (40 to 50 ounces), reaching a maximum two or three hours after a large fluid intake. Women excrete somewhat less than men, while children void relatively more than do adults, although the actual amount is less. In the adult we find the amount of urine is almost directly proportional to his weight, a normal large individual excreting nearer 1,800 than 1,500 c.c., the amount being about 1 c.c. per kilo and hour, while with a child the excretion is about 4 c.c. per kilo and hour.

The physiologic limits of the urinary excretion are about 750 and 3,000 c.c. In cases showing as high an output as 3,000 c.c. one is justified, perhaps, in assuming the presence of some pathologic condition. The kidneys are not easily deranged by the excess work put upon them in excreting large quantities of urine, so that we may find a secretion of many liters per day continuing for an extended period without endangering the normality of the excreting organ.

Normally, the amount of urine excreted during the day by far exceeds that voided during the night, while the afternoon urine is usually more than that of the morning. We find, however, that in edematous conditions either of hepatic, cardiac, or renal origin, the night urine usually exceeds that of the day. This condition is known as *nycturia*. As it is, perhaps, more frequently associated with cardiac insufficiency, it may have a diagnostic importance in these cases.²

Polyuria.

By this is meant an excretion of an increased amount of urine. Just what amount of urine is to be considered as indicative of polyuria will depend much upon the habits of the patient as regards daily intake of fluid. As a rule, anything above 2,500 c.c. is at least suggestive of this condition.

Just what factors are to be held accountable for the polyuria, is not always easy to decide in every case. An increased intake of fluid together with an increased general blood-pressure will cause both an increased local renal pressure and an increased blood-flow through the kidney. While such a polyuria rarely exists in cases of ordinary chronic or active renal hyperemia, we find under the influence of drugs that a very decided increase in the urinary output may occur.³

A polyuria is observed in the convalescent stages of acute nephritis, in

¹ See Addis and Watanabe, Jour. Biol. Chem., 1916, XXVII, 267; Haldane and Priestley, Jour. Physiol., 1916, L, 296; Priestley, Ibid., 304.

² See Jespersen, Hospitaltid., 1916, LIX, 1229; Barach, Am. Jour. Med. Sc., 1921, CLXI, 551; Zak, Med. Klin., 1921, XVII, 1193; Campbell and Webster, Biochem. Jour., 1921, XV, 660; Jones, Jour. Am. Med. Assoc., 1922, LXXVIII, 477.

³ See Cushny and Lambie, Jour. Physiol., 1921, LV, 159.

both chronic parenchymatous and interstitial nephritis, and in amyloid degeneration of the kidney. This excretion, especially in the chronic interstitial type, may be one in which the total solids are normal or reduced, and is then known as *hydruria*.

Diabetes mellitus is more frequently, perhaps, than other conditions associated with a polyuria. The quantity eliminated is dependent both upon the increased intake as a result of the polydipsia as well as upon the dehydrating powers of the sugar. A certain relationship exists between the amount of fluid and the sugar, the polyuria being usually diminished by measures which decrease the amount of sugar excreted. This polyuria is not necessarily continuous and may alternate with periods showing a normal or subnormal amount of urine.

In cases of diabetes insipidus we find the daily excretion of as much as 50 liters or more of urine. According to Meyer, this polyuria is due to the attempt on the part of the kidneys to secrete sufficient water to hold the solids in solution.¹

In cases associated with abnormal accumulations of fluid, such as pleuritis, ascites, and general edema, a polyuria will exist at the time of absorption of the exudates, owing to the presence of such large amounts of fluid in the blood-vessels.²

The so-called "epicritic polyuria" is frequently observed during convalescence from acute febrile attacks. This is probably indicative of the elimination of toxic products which have accumulated in the system during the progress of the disease. It is supposed to be of favorable import when occurring in a febrile condition, but it is to be recalled that this polyuria may be followed by a later oliguria which is of grave significance. As a rule, however, it may be said that as the case improves the urine is increased in amount.

Polyuria may be observed in many nervous conditions, both functional and organic.³ The cause is probably some disturbance of the vasomotor

¹ See Lewis and Matthews (Trans. Chic. Path. Soc., 1913, IX, 16) who show the close relation of hypersecretion of the pars intermedia of the hypophysis to diabetes insipidus. See Socin, Ztschr. f. klin. Med., 1913, LXXVIII, 294; also, Forschbach, Ibid., 1913, LXXVII, 153; Goldzieher, Verhandl. d. deutsch. path. Gesellsch., 1913, XVI, 272; Berblinger, Ibid., 281; Simmonds, Münch. med. Wchnschr., 1914, LXI, 180; Hohlweg, Ibid., 927; Fitz, Arch. Int. Med., 1914, XIV, 706; Mathews, Ibid., 1914, XV, 451; Krikortz, Hygiea, 1915, LXXVII, 49; Motzfeldt, Boston Med. & Surg. Jour., 1916, CLXXIV, 644. Jour. Exper. Med., 1917, XXV, 153; Rosenbloom and Price, Am. Jour. Dis. Child., 1910, XII, 53; Newmark, Arch. Int. Med., 1917, XIX, 550; Christie and Stewart, Ibid., XX, 10; Oehme, Deutsch. Arch. f. klin. Med., 1918, CXXVII, 261; Leschke, Ztschr. f. klin. Med., 1919, LXXXVII, 201; Kennaway and Mottram, Quart. Jour. Med., 1919, XII, 225; Marañon and Gutierrez, Sig. Méd., 1919, LXVI, 800; Lereboullet, Progrès Méd., 1919, XXXIV, 363. Oehme, Ztschr. f. exp. Med., 1919, IX, 251; Pagniez, Presse méd., 1919, XXVII, 746; Schnabel and Gerhard, New York Med. Jour., 1920, CXI, 812; Gorke, Arch. f. Verd.-Kr., 1920, XXVI, 365; Strauss, Deutsch. med. Wchnschr., 1920, XLVI, 930; Nasso, Pediatria, 1920, XXVIII, 812; Schulmann and Desoutter, Rev. de Méd., 1920, XXXVII, 441 and 520; Evans and Wallis, Lancet, 1921, I, 70; Winslow, Northwest Med., 1921, XX, 16; Maranon, Endocrinology, 1921, V, 159; Villa, Policlinico, 1921, XXVIII, 438; Rabinowitch, Arch. Int. Med., 1921, XXVIII, 355; Bailey and Bremer (Ibid., 773) believe that the hypothalamus is involved in this polyuria; Weir, Larson and Rowntree, Ibid., 1922, XXIX, 306; Christie and Stewart, Ibid., 555.

² See Baehr, Deutsch. Arch. f. klin. Med., 1913, CIX, 417; also, Leuret, Jour. de méd. de Bordeaux, 1913, LXXXIV, 669.

³ See Pirondini, Jour. d'Urol., 1914, V, 461; Mathieu, Bull. Acad. de Méd., 1915, LXXXIII, 208.

apparatus as a result, perhaps, of irritation of the floor of the fourth ventricle, cerebellum, or cord. Hysteria, neurasthenia, epilepsy, and chorea are frequently associated with a polyuria. A paroxysmal polyuria in the course of a suspected nervous disease is more indicative of a functional derangement, while a continuous polyuria is more frequently associated with true organic disease.

Oliguria.

This is a condition characterized by the excretion of a diminished amount of urine, 800 c.c. being given as the lower normal point of the urinary output. Here, again, the absolute figure must depend upon the patient and upon his customary excretion. A single examination is not sufficient to decide whether or not an oliguria exists.

This condition is found, perhaps, most frequently in cases of broken compensation of the heart, where the blood-pressure is markedly diminished. It is present whether the cardiac incompetency be primary or secondary to hepatic, renal, or pulmonary lesions.

Oliguria is noted in practically all acute febrile disorders, especially in typhoid fever. This is due, probably, to a combination of cardiac weakness with the increased loss of water by the skin and lungs. Moreover, we may have, in such states, a retention of fluid along with a direct contraction of the renal vessels.

Acute nephritis as well as chronic parenchymatous nephritis are associated with a more or less extensive oliguria. This condition is probably referable to diminished functional activity of the glandular elements as well as to increased resistance within the tubules. A bilateral diffuse lesion is always necessary to cause much oliguria, as the sound kidney, if the trouble be unilateral, will take on vicarious activity. The more acute the condition, the greater the degree of oliguria.

Oliguria may also occur following the administration of an anesthetic, in connection with eclampsia, hysteria, or epilepsy, after the loss of large quantities of fluid by hemorrhage, diarrhea, or vomiting, in cases of portal obstruction as seen in acute yellow atrophy or hepatic cirrhosis, or in cases in which pressure is exerted upon the vascular system, especially the vena cava, by tumors.¹

Anuria.

This oliguria may, in almost any case, proceed to complete anuria, which may or may not be of vital significance. Cases of anuria do occur without any preceding oliguria, as shown in acute nephritis and in some cases of hysteria. Anuria, *per se*, cannot be held responsible, however, for the uremic symptoms so frequently associated with it, as it may persist for many days, 19 in a case of Adams, without any uremic signs.

Anuria may be due to obstructive, reflex, renal, and prerenal causes. We

¹ See Cohn, Berl. klin. Wchnschr., 1915, LII, 208; von Szöllösy, Ztschr. f. exper. Path. u. Therap., 1915, XVII, 243, calls attention to the importance of opsiauria (a retardation of the urinary output) from the diagnostic standpoint. See Cottet, Médecine 1921, II, 467.

may have an occlusion of the urinary passages¹ on one side and a reflex closure on the other. Tumors, prostatic hypertrophy, and toxic and nervous bladder disturbances may lead to a great degree of oliguria amounting, almost, to anuria.

The so-called prerenal causes of anuria include scarlet fever, which may lead to a severe nephritis, phosphorus poisoning, action of ether and chloroform, collapse, ureteral and urethral calculus, and cholera.

(2) Appearance.

Freshly voided urine should be clear and transparent. Only the faintest trace of any turbidity should be normally present, except soon after a meal rich in vegetable food, when a distinct turbidity may be noticed due to the precipitation of the phosphates in the alkaline urine.

When allowed to stand for a short time, a light cloud is noted which gradually settles to the bottom of the container in the form of the so-called "nubecula." This contains a few small granular cells and a few epithelial cells and is composed largely of mucus.

On standing for a somewhat longer period, as for instance over night, at the ordinary temperature, distinct crystals of uric acid may separate and appear in the sediment. If the temperature of the urine is allowed to fall to a considerable extent during this period, a somewhat more marked turbidity will be produced owing to the precipitation of the acid urates. This sediment is particularly noticeable if a highly acid urine becomes very cold.

If kept for a longer period at room temperature, or a shorter period during the warmer months, a diffuse cloudiness will appear, due to the precipitation of the phosphates, owing to the lessened acidity or abnormal alkalinity of the urine. This alkalinity is due to the decomposition of the urea into ammonium carbonate. The crystals in this alkaline urine will be triple phosphates, calcium phosphates, ammonium urate, and calcium carbonate.

Even before the urine becomes alkaline, a diffuse cloudiness may be present, due to the development of numerous saprophytic bacteria. This bacterial cloud is removed only with the greatest difficulty, as filtration of the urine has practically no effect upon it. Frequently, the addition of lead acetate to the urine will produce a voluminous precipitate, which may carry down the bacteria and permit of their filtration. This procedure is, however, not to be recommended, as other substances, if present in small amounts, may be carried down and thus escape detection.

If the urine is cloudy when freshly voided, the turbidity may be the result of the precipitation of phosphates through the alkalinity or it may indicate the presence of an organized sediment, such as casts, epithelial cells, blood, and pus.²

The normal urine shows but very little viscosity, differing little from

¹ See MacNider (Jour. Med. Research, 1912, XXVI, 79) for a discussion of the relation of swelling of the tubular epithelium to the urinary output. Also, Cecil Jour. A. M. A., 1917, LXVIII, 440; Sala Paliclinico, 1920, XXVII, 1242. Scholl and Foulds (Jour. Am. Med. Assoc., 1921, LXXVI, 368) report an elaborate study of the functional findings in a case of prolonged anuria.

² Shaw (Proc. Roy. Soc., Med., Sect. Med., 1920 XIII, 105) reports an unusual turbidity due to the presence of lecithin-globulin.

ordinary water in this respect.¹ In certain conditions we find a marked degree of viscidty, which becomes especially apparent on attempting to filter the urine. In cases of chronic cystitis the excretion of a large amount of mucus may make the urine ropy and gelatinous.² This increased viscosity may also be seen in cases of pyuria associated with decomposition.³

(3) Color.

The color of the urine varies normally between various shades of yellow, the depth of color depending upon the concentration or specific gravity of the specimen. While the color is usually much paler in the urines of low specific gravity and very dark in those of high density, we find in diabetes mellitus a very pale urine with a high specific gravity. In cases of anemia the urine is always paler than normal, but in pernicious anemia the urine is highly colored owing to the marked destruction of the erythrocytes.

As a rule, it may be said that an acid urine is more highly colored than an alkaline one, although many exceptions to this rule occur. There seems to be some difference between the urines passed at different periods of the day; thus the urine of the day is usually a distinct amber, while that of the night may take on a greenish tinge. To what this color in the latter case is due is at present unsettled. Several color scales have been introduced, such as those of Neubauer and Vogel and of Radde, but these are not sufficiently extensive to take in pathologic variations where they would be most important. As a rule, it is sufficient to divide the colors of the urine into those of the spectrum, making allowance for light, medium, and dark shades of each color.

The pigments causing the normal and abnormal colorations of the urine will be discussed in detail in a later section. At this point the writer would say that normally these pigments are urochrome, uroerythrin, and urobilin, while the various conjugated glycuronic and sulphuric acids, blood pigments, biliary pigments, melanin, etc., are found in pathologic conditions.

Pathologic Colorations.

Deviating from the rule that the higher the specific gravity the more intense the color, diabetes mellitus shows an extremely light color with a high specific gravity. Owing to the lack of pigment, in chlorosis we find a very pale urine. Chronic interstitial nephritis and amyloid degeneration of the kidneys are associated with an extremely pale urine.

In febrile conditions the coloration may range from an orange-red to a distinctly red tint, owing to the increase in the amount of urobilin. This deep color is especially noticeable in cases of severe pneumonia.⁴ This reddish urine may also be due to an increase in the amount of uroerythrin, which is responsible for the deep color of the urate sediment so frequent in the concentrated urines of febrile conditions, circulatory disturbance of the liver, and in cases associated with profuse perspiration.

¹ See Posner, *Berl. klin. Wchnschr.*, 1915, LII, 1106.

² See Tocco, *Polichinico*, 1921, XXVIII, 1548; Capogrossi, *Ibid.*, 1724; Jose *Biochem. Ztschr.*, 1921, CXIX, 93.

³ See Steensma (*Nederl. Tijdschr. v. Geneesk.*, 1914, LVIII, 24), who reports "fluorescence in certain types of urines.

⁴ See Hildebrandt, *Ztschr. f. klin. Med.*, 1911, LXXIII, 189.

In cases of jaundice the urine may vary from a dark yellow or green to a brown or black, depending upon the concentration of the urine, the amount of bile pigments, and upon certain chemical activity which occurs in such urine. Not only will the color of the urine be deeper, but the foam which appears on shaking the specimen will take on a distinct yellowish-brown tint. If this biliary urine be allowed to stand in the cold for some time, crystals of bilirubin may separate out and be seen in the sediment.¹

Urine which contains blood may have a violet shimmer, may appear smoky, blood red, brownish-black, or even deep black in color. These variations depend both upon the amount and kind of pigment present. Hemoglobin gives a more reddish tint to the urine, while methemoglobin produces a brownish shade. Such urine is always cloudy, owing to the admixture of corpuscles and other organic material. The blood found in the urine may arise from any point in the genitourinary tract or may be of systemic origin. In the latter case conditions which give rise to hemolysis will cause the appearance of hemoglobin in the urine.

The condition of *chyluria* is characterized by the presence of large numbers of highly refractile globules of fat along with many morphological constituents. This gives rise to the appearance of a milky urine and is especially characteristic of infection with the filaria. It is not infrequent to find, in certain cases of hysteria, a specimen of milky urine, owing to the fact that the patient has added milk to the urine before sending it to be examined. The presence of a large quantity of pus will also give the urine a milky appearance.

The urine of patients suffering with melanotic tumors may be perfectly clear when freshly voided, but becomes black or dark brown on exposure to the air. This reaction is due to the transformation of the pigment melanogen to melanin, and may be hastened by the addition of oxidizing agents to the urine. This darkening of the urine extends characteristically from above downward.

The condition known as *alkaptonuria*, which is characterized by the excretion of homogentisic and uroleucic acids, gives rise to the passage of a urine which is brownish-black in color and may be syrupy in consistency. This color is not always evident in the fresh specimen, but appears soon after being voided.

In cases of peritonitis, suppuration anywhere in the system, gangrene, and marked intestinal putrefaction, the urine is frequently dark colored owing to the passage of certain aromatic products of decomposition, either indican or various derivatives of phenol. The coloration in these cases may vary from a dark brown or greenish-black to a distinct blue. These urines differ from those containing melanin in the fact that ferric chlorid does not blacken the urine as it does in the presence of melanin. This urine may contain a distinct amount of indigo, although the substance present is usually a different oxidation product. If indigo be present, a bluish-black scum will frequently rise to the surface of the specimen.

Medicinal Coloration.

After the use of carbolic acid either internally or externally, guaiacol,

¹ See Dorner, Deutsch. med. Wchnschr., 1922, XLVIII, 453.

creosote, resorcin, naphthalin, salol, and various tar preparations the urine may vary from a dark brown to a black color. This coloration is due to the excretion of hydroquinon and of pyrocatechin, and may be evident only on allowing the urine to stand for some time. The urine containing pyrocatechin may reduce alkaline copper solutions, but will not affect such bismuth preparations. While a dark brown or black coloration of the urine may be found in cases of hemorrhage, melanosis, malaria, alkaptonuria, ochronosis, and chronic tuberculosis, one should be on his guard, as the medication of the case may be responsible for such coloration.

Methylene blue will color the urine a greenish to deep blue shade, which may last for several days. Usually within an hour after this drug is taken the urine may show a faint tinge of green which may be more clearly brought out by acidifying with acetic acid and warming.

The use of the hypnotics, trional, sulphonal, and tetronal, frequently gives rise to the voiding of a urine which has a deep red-wine color, due to the presence of hematoporphyrin. Pyramidon produces a urine of rose-red color, the pigment of which is soluble in ether, chloroform, and amyl alcohol. Antipyrin and purgatin both produce distinctly red urines. Chrysarobin, senna, rhubarb, cascara, and santonin produce a golden-yellow urine which becomes red in the presence of alkali. This coloration is due to the excretion of chrysophanic acid. According to Gorup-Besanez, the pigment of beets, huckleberries, blackberries, etc., may under certain conditions be excreted in the urine and color it the corresponding shades.

(4) Odor.

The normal urine usually has a distinct aromatic odor which very much resembles that of beef broth. This odor is due to the presence of certain volatile acids and is more marked in urines of high concentration.¹ If the urine undergoes decomposition either within the bladder or on standing, a so-called "*urinous odor*" appears which is due to the decomposition of protein material. This odor is very markedly ammoniacal. Should such an odor appear in the freshly voided specimen, it is evidence of marked cystitis.

Abnormal decomposition of the urine, as evidenced by changes in the odor, may be found in conditions associated with decomposition of pus and may be due to the presence of hydrogen sulphid along with the ammonia. This condition may be observed in cases of perforation of an abscess into the urinary tract, in which case the urine may have a distinctly fecal odor if the intestine be involved, while in carcinoma of the bladder this repulsive odor of the urine may also be noticed.

A distinct fruity odor is often present in cases of diabetes mellitus, in many febrile conditions, and in some stomach and intestinal troubles, which may be directly traceable to the presence of acetone.

Certain medicaments, such as oil of turpentine, give rise to a distinct odor of violets in the urine. Menthol causes an odor of peppermint, while cubebs, copaiba, sandal-wood oil, tolu, and saffron produce a peculiar spicy

¹ Dehn and Hartman (Jour. Am. Chem. Soc., 1914, XXXVI, 2118 and 2136) believe the odor of urine to be due to a peculiar substance which they call "urinod." See, also, Hartman, Arch. Int. Med., 1915, XVI, 98.

odor. Valerian and asafetida are excreted as such in the urine and produce their characteristic odor. Certain foods, such as meat, bouillon, and coffee, produce a slight odor of the urine, while asparagus gives a peculiar characteristic odor due to the presence of methyl mercaptan.

(5) Reaction.

The normal urine has an acid reaction. According to the views formerly held, this acidity was directly due to the presence of acid salts, especially to sodium dihydrogen phosphate (NaH_2PO_4), and not to the presence of any free acid.

The recent work of Folin has shown that the phosphates in the clear urine are all of the monobasic (diacid) type. His figures indicate that the acidity of normal clear urines is ordinarily greater than the acidity of all the phosphates present and that the excess must be due to free organic acids. For this reason the methods of Freund and of Lieblein for the determination of the acidity of the urine must be given up. To quote from Folin:¹ "The current attractive, and in a measure plausible, belief that the acidity of urine is regulated by variations in the relative proportion of the two forms of 'acid phosphates' is, therefore, erroneous. If urine does at no time contain comparatively strong acids in the free form, the reason is in part the variability of the ammonia formation and in part the presence of salts of organic acids. In a mixture of salts containing an excess of acids it is the weakest which will remain uncombined and the strongest organic acids will, therefore, exist as salts; but if the total amount of acidity becomes abnormally great, the quality (the strength) of the free acids may change."

From the standpoint of physical chemistry the acidity of the urine, as of all other acid solutions, should represent the absolute number of dissociated hydrogen ions in a definite quantity of the urine.² We are, therefore, face to face with the same problem confronting us in the examination of the alkalinity of the blood. In the case of the urine the question of indicator to be used in the titration test is a matter of great moment, as no two indicators will give the same degree of acidity. The one naturally to be selected would be that which will react to every possible substance of an acid nature.

If we use the methods of physical chemistry we find, according to Höber,³ that the urine is only about 30 times as acid as is distilled water and only about one ten-thousandth as acid as the titration figures would indicate. Such being the case, we must either entirely revise our figures for the acidity of the urine or employ methods which can be more easily carried out by the general worker than can those of physical chemistry.⁴

¹ *Am. Jour. Physiol.*, 1905, XIII, 45.

² For a consideration of methods for determining the H-ion concentration see Chapter on Blood. The P_{H} values for urine are, of course, subject to wide variation under different influences but the usual figures run from 5.98 as reported by Henderson and Palmer (*Jour. Biol. Chem.*, 1912, XIII, 393 and 1914, XVII, 305) to 6.64 as shown by Blatherwick, *Arch. Int. Med.*, 1914, XIV, 409.

³ *Beitr. zur chem. Physiol. u. Path.*, 1903, III, 525.

⁴ See Henderson and Palmer, *Jour. Biol. Chem.*, 1913, XIII, 393; *Ibid.*, 1913, XIV, 81, *Arch. Int. Med.*, 1913, XII, 153; also, Henderson, *Science*, 1913, XXXVII, 389; Henderson; Palmer and Newburgh, *Jour. Exper. Pharmacol.*, 1914, V, 449; Henderson and Palmer, *Jour. Biol. Chem.*, 1914, XVII, 305; *Ibid.*, 1915, XXI, 37; *Ibid.*, 57; *Arch. Int. Med.*, 1915, XVI,

Folin has, therefore, introduced a method which uses direct titration of the urine and employs phenolphthalein as an indicator. This indicator reacts to all bodies of an acid nature, but cannot overcome certain difficulties which are in the way of direct titration. These obstacles are (1) the occurrence of calcium in the urine in the presence of the monobasic phosphates, and (2) the presence of ammonium salts. He has found that the addition of potassium oxalate to the urine will do away with these difficulties by holding in solution both the *di-* and *tri-calcium* phosphates and by preventing the dissociation of the ammonium compounds.

Folin's Method.

Total Acidity.

Twenty-five c.c. of urine are treated with 15 to 20 grams of powdered potassium oxalate and one or two drops of a 1 per cent. alcoholic solution of phenol-phthalein. The mixture is shaken rapidly for one or two minutes and titrated at once with a tenth-normal sodium hydrate solution until a faint, distinct, permanent pink color is obtained. It is advisable to shake the flask during the titration so as to prolong the effects of the potassium oxalate. The acidity is expressed in terms of the amount of tenth-normal sodium hydrate solution necessary for neutralization of the 24-hour amount of urine. This is expressed as T, which is, on an average, 617.

The studies of Fitz and Van Syke on acid excretion were undertaken to ascertain whether a quantitative relationship could be discovered between the alkaline reserve of the blood plasma, as measured by its combining power for CO₂, and the rate of acid excretion by the kidneys. It has been known for some time that entrance of acid into the circulation immediately reduces the blood bicarbonate and is accompanied by an increased rate of acid and ammonia excretion in the urine. In this work the plasma bicarbonate is estimated by the CO₂ combining power method of Van Slyke; the ammonia by the permutit method of Folin and Bell (as discussed on page 254); and the acid titratable with phenolphthalein as an indicator by the method of Folin given above. This latter was selected because the acid titratable with phenolphthalein approaches zero in human urine when the height of the plasma bicarbonate is at its maximum normal of about 80 volumes per cent., under which conditions ammonia excretion also approaches zero. These workers present their results in the form of an equation, comparable to those of Ambard and of McLean for the urea and chlorid relations of the blood and urine (as discussed later), which permits of the determination of the relationship of this excretion to the alkaline reserve of the body as measured by the CO₂ combining power of the blood plasma. The original formula of Fitz and Van Slyke for this relationship is as follows:

$$\text{Plasma CO}_2 \text{ Capacity} = 80 - \sqrt{\frac{D}{W}}\sqrt{C}, \text{ in which D represents the rate}$$

199; Höst, Ztschr., f. klin. Med., 1915, LXXXI, 266; Van Slyke and Palmer, Proc. Soc. Exper. Biol. and Med., 1919, XVI, 140; Haskins, Jour. Lab. and Clin. Med., 1919, LV, 363; Talbert, Am. Jour. Physiol., 1920, L, 579; Morató, Siglo Méd., 1921, LXVIII, 290; Rohonyi, Ztschr. f. klin. Med., 1921, XCI, 105; Lyon and Trager, Med. Record, 1922, CI, 543; Marshall, Jour. Biol. Chem., 1922, LI, 3.

of excretion of 0.1 N ammonia plus 0.1 N titratable acid per 24 hours; C the 0.1 N ammonia plus 0.1 N acid per liter of urine, and W the body weight. The index may be determined from analysis of the urine passed in 24 hours or from the amount excreted in 1 or 2 hours, multiplied to bring the data to a 24 hours basis. From the work of Barnett, it is evident that the average values for the fourth root of C is 5. Hence the above formula may be simplified as follows without impairing the general accuracy:

$$\text{Plasma CO}_2 \text{ Capacity} = 80 - 5 \sqrt[4]{\frac{D}{W}} = \text{Index of Acid Excretion.}$$

As Stillman, Van Slyke, Cullen and Fitz show, the urine index in very severe acidosis is less accurate than the alveolar air in indicating the alkaline reserve. With a plasma CO₂ of 25 per cent., which corresponds to a urine index of 55, the index may be 65, indicating 15 per cent. plasma CO₂, which is fatal in their experience, or it may be 45, indicating 35 per cent. plasma CO₂, which, though a pronounced acidosis is so well above the danger limit that signs of coma are usually absent. The alveolar carbon dioxide method appears the more accurate in measuring the more severe stages of diabetic acidosis, while the index of acid excretion is the more accurate in measuring the more common intermediate stages. The normal values for this index range from 3 to 27. Anything above 27 indicates an acidosis, which usually becomes critical if it approaches 100 c.c. per kilo. It is to be remarked that the results by this method do not agree within approximately 10 per cent., so that deviations of this amount either way must be allowed for.¹

Free Mineral and Organic Acidity.

Determine the amount of total phosphates present by titration with uranium nitrate solution as described later. Seven and one one-hundredth mg. of P₂O₅ have an acidimetric value equal to 1 c.c. of tenth-normal acid. The total acidimetric value of the phosphates of the 24-hour urine may be easily determined with the help of this factor, by converting the amount of phosphates into terms of N/10 acid.

From the total acidity (T) subtract the acidimetric value of the phosphates (P). The remainder is the acidity due to uncombined organic acids, and the difference, that obtained from calculating all the phosphoric acid as diacid phosphate, is the free mineral acidity. For all ordinary studies of the acidity of the urine the direct titration of the total acidity and of the phosphates gives the necessary information.² The excess of the total acidity above that calculated from the phosphates gives the total free acids present. If the acidity calculated from the total phosphates is greater than the titrated acidity, then there are practically no free organic acids present, and the titrated acidity represents the amount of phosphates present in the diacid form (Folin).

¹ Fitz and Van Slyke, Jour. Biol. Chem., 1917, XXX, 389; Stillman, Van Slyke, Cullen, and Fitz, Ibid., 405; Barnett, Ibid., 1918, XXXIII, 267; Van Slyke, Ibid., 271; Van Slyke and Palmer, Ibid., 1920, XLII, 567; Palmer, Salvesen and Jackson, Ibid., 1920, XLV, 101.

² See Walpole (Biochem. Jour., 1914, VIII, 628) for a discussion of various new indicators.

While the acidity of the urine is best determined and expressed as outlined above, it seems wise to the writer to retain the same style of expression for the acidity as used in stomach analysis. With this nomenclature one would state the acidity of the urine in degrees, that is, the amount of tenth-normal sodium hydrate necessary to neutralize 100 c.c. Under normal conditions this will vary from 35 to 45°. It may be increased by a diet rich in meat, while it is decreased by a vegetable diet. There are many acids produced in the oxidation of protein, among which we find sulphuric, phosphoric, uric, and the oxyaromatic acids. Ordinarily, these play an indirect part in the acidity of the urine, although this phosphoric acid may exist in part as the dihydrogen phosphate and in consequence increase the acidity of the urine. The regulation of the metabolism is such that an increase of the acids produced in the system or taken into it from without is neutralized by an increased formation of ammonia, the salts appearing in the urine as the ammonium salts which do not, of course, increase the acidity of this fluid. This is the basis upon which one estimates the amount of ammonia in following a condition of acidosis.¹

The reaction of the urine varies at different times of the day. The acidity appears to be highest in the morning before breakfast and is diminished after a meal, due to the secretion of hydrochloric acid into the stomach. The reaction of the urine may even be alkaline for a period of two or four hours after each meal, in which case the urine will be turbid from the precipitation of phosphates. This reaction of the urine following meals is known as the "alkaline tide" of the urine. Between meals the acidity of the urine will gradually increase until the next meal is taken.²

The reaction of the urine is modified to a great extent by the use of drugs.³ Thus, alkalies, such as carbonate and bicarbonate of sodium, will render the urine alkaline if taken between meals, while if taken just preceding a meal they will be neutralized by the gastric juice. All organic acids of the fatty series are oxidized in the system to carbonic acid and combine with bases forming basic salts which render the urine alkaline or less acid, providing these acids are not taken above the point of tolerance, as the writer has shown that large doses of such acids as citric acid will increase the acidity of the urine. The mineral and aromatic organic acids will, however, practically always increase the acidity.⁴

In many pathologic conditions we find the reaction of the urine variable.⁵ Abnormal gastric activity may be either associated with an increase or a

¹ Fischer (Nephritis: An Experimental and Critical Study of Its Nature, Cause and the Principles of Its Relief, New York, 1912) believes that the abnormal production or accumulation of acid in the cells of the kidney and the action of this acid on the colloidal structures of the kidney are accountable for all the changes which characterize nephritis. See, also, Hirschfelder, *Jour. A. M. A.*, 1916, LXVII, 1891.

² See Fiske, *Jour. Biol. Chem.*, 1921, XLIX, 163; *Ibid.*, 1922, LI, 55.

³ See de Jager (*Biochem. Ztschr.*, 1912, XXXVIII, 294), who shows that magnesium sulphate increases the acidity, while sodium sulphate diminishes it. Holló (*Biochem. Ztschr.*, 1921, CXIII, 246) believes that phosphates increase the acidity.

⁴ See Stehle and McCarty, *Jour. Biol. Chem.*, 1921, XLVII, 315; Campbell and Webster, *Biochem. Jour.*, 1922, XVI, 106.

⁵ See Newburgh, Palmer and Henderson, *Arch. Int. Med.*, 1913, XII, 146; also, Pertik (*Virchow's Arch. f. path. Anat.*, 1913, CCXIII, 465) who finds a decreased acidity in tuberculosis; Labbé and Vitry, *Presse Méd.*, 1914, XXII, 437.

decrease in the acidity of the urine, depending upon a condition of hypo- or hyperacidity of the gastric juice. The rapid absorption of a transudate or exudate will lead to the excretion of an alkaline urine from the presence of an increased amount of alkaline salts. An alkaline urine is not infrequently seen after intestinal hemorrhage, in certain cases of pneumonia, typhoid fever, chronic nephritis, and in cases in which exudates from the urinary tracts have become mixed with the urine. In certain cases of nervous diseases and in some cases of anemia we may also find an alkaline urine. The urine in all of the above cases will show, if tested by litmus-paper, an alkaline reaction in which the bluing of the red litmus-paper is permanent. This condition is known as *fixed alkalinity* and is quite distinct from the following type.

In cases of decomposition of the urine within the urinary tract, through the influence of bacteria, the urea is decomposed into ammonium carbamate and carbonate. The alkaline reaction of the urine in such cases will be shown by a blue color of the red litmus-paper either held above it or placed in it, the blue color disappearing when the paper is dried. This condition is known as *volatile alkalinity*. If the urine shows this volatile alkalinity on being voided, the finding is significant of trouble somewhere along the urinary tract, especially within the bladder.¹

Benedict has recently introduced the term "acid unit" into the study of the urinary output. One c.c. of urine with an acidity of 1 degree is equivalent to 100 acid units. In other words, one may determine the acid units in the 24-hour specimen by multiplying the number of cubic centimeters of the urine by the degrees of acidity. This is normally about 40,000.

(6) **Specific Gravity.**

The specific gravity of the normal urine ranges between 1,015 and 1,025, with an average of 1,020. This specific gravity will depend, of course, upon the amount of fluid intake, the quantity of the 24-hour specimen of urine, the degree of tissue activity, and the condition of the secreting organs. The intake of a large volume of water may reduce the specific gravity of the urine to a very low figure and, correspondingly, a small intake may lead to a urine of high gravity. We find, therefore, that perfectly normal urines may show specific gravities ranging from 1,010 to 1,030, with pathologic variations from 1,002 as high as 1,060 or more.²

In general routine work it is essential that the specific gravity of the 24-hour specimen be determined. Except in unusual cases, absolutely nothing of diagnostic value may be learned by the determination of the specific gravity of a single voiding of urine. The variations at different times of the day, under the influence of food, digestion, activity of the skin and lungs, and exercise, may be so great that apparent pathologic figures may be obtained from a single specimen. Such variations are overcome for the most part in the 24-hour specimen unless pathologic conditions are present to keep up such variations. In some cases, especially in chronic diffuse nephritis, the morning specimen of urine almost invariably has a lower specific gravity

¹ See Saidman, C. R. *soc. biol. de Paris*, 1916, LXIX, 780.

² See Frey, *Deutsch. Arch. klin. Med.*, 1912, CVI, 347.

than that of the other periods of the day. For this reason one may determine the specific gravity of a single specimen of such urines.

It is of especial importance that the total amount of urine in the 24-hour specimen be taken into consideration in judging of the value of a specific gravity. Thus in chronic interstitial nephritis we may find a large volume of urine with a low specific gravity, while in diabetes mellitus an even higher volume of urine may be present, showing a very high specific gravity.

Technic.

The most accurate method of determining the specific gravity is, of course, the use of the pycnometer. The principle of this method is the determination of the weight of a definite volume of urine as compared with that of the same volume of distilled water under the same conditions of temperature and atmospheric pressure. This method will be discussed in the section on Blood, to which the reader is referred.

The clinical method of estimating this factor is a distinctly areometric one. The principle of this method is that a body immersed in a fluid will displace an amount of fluid equivalent to the loss in its own weight. By the use of instruments known as hydrometers or in the case of the urine as urinometers, this displacement is measured by immersing the hydrometer in the fluid and observing the point to which this instrument sinks. The stem of the hydrometer is graduated in divisions from 1,000 to 1,060 by differences of 1° , the 1,000 point being that to which the instrument sinks when immersed in distilled water at the temperature to which the instrument is calibrated. Any variation in the density of the solution in which this hydrometer is immersed will be evident by the depth to which it sinks, the more concentrated the solution the less will the instrument sink.

The vessel in which the urine is poured should be cylindrical in shape, with parallel sides and wide base and sufficiently tall to permit of the complete sinking of the hydrometer. The forms of this cylinder with fluted sides are perhaps more desirable than the plain cylinder, as the bulb does not tend to stick to the sides of the vessel so readily.

The vessel is filled about four-fifths full of urine, any foam being removed by the use of filter-paper. The hydrometer is placed in the urine with a twisting motion and allowed to come to rest. The depth to which the stem is immersed is then read off by observing the mark which coincides with the lower meniscus of the urine as seen from below. The worker should never attempt to read the specific gravity from above as a slight meniscus interferes with the accuracy of his reading. The worker must be absolutely sure that the urinometer neither rests upon the bottom of the cylin-



FIG. 70.—Urinometer and cylinder. (Hawk.)

der nor touches the sides, but should see that it floats perfectly free in the urine.

The temperature at which the reading is taken is a matter of some moment as some of these instruments are graduated at 15°C . The ordinary model, as made by Squibb, is graduated at 25°C ., which is, perhaps, more nearly the working temperature of the room. A variation of 3° in temperature between that of the room and that at which the instrument is calibrated, will give a difference of 1° of specific gravity; that is, a difference in the fourth place of the specific gravity. In ordinary clinical work corrections for variations in temperature are usually unnecessary, as variations of two or three points in the fourth place of the specific gravity are of absolutely no importance, as such changes might be attributed to chemical variations on standing, even though the most accurate methods of estimating the specific gravity were used.

If the quantity of urine be very small, it may be diluted with distilled water, so that the measuring cylinder may contain enough material to permit of a density estimation.¹ The specific gravity of this diluted urine is then determined as above and the last two figures of the specific gravity are multiplied by the degree of dilution.

Rough Estimate of Total Solids.

As the degree of specific gravity is directly proportional to the amount of solids contained in the urine, one may roughly judge of the total solids by a simple calculation as follows: If the last two figures of the specific gravity be multiplied by 2.33 (Haeser's coefficient), the result will be the approximate number of grams of total solids in every 1,000 c.c. of urine. Knowing the quantity of urine passed in the 24 hours, a simple calculation will yield the total 24-hour excretion of solids. Long uses the coefficient 2.6. Instead of the above figure one may multiply the last two figures of the specific gravity by 1.1 (Haines' coefficient) and obtain the number of grains of solids in each fluidounce of the urine. This figure when multiplied by the total number of ounces of the 24-hour specimen will yield the excretion of solids in grains. This latter method has some advantage for the older practitioner who has not accustomed himself to the use of the metric system.

It is to be said that neither one of the above methods can give anything but approximate results and in pathologic urines are absolutely unreliable. Highly albuminous urines invariably show a reduced specific gravity, while a high sugar content is associated with an increase in the density. In either one of these cases a calculation of the total solids by the above method will yield inexact figures. If the worker desires to know the exact amount of total solids in the 24-hour specimen, and this is sometimes advisable, recourse should be had to more exact methods of determination which will be discussed later.

The specific gravity of a specimen of urine varies, of course, with the

¹ Noeggerath and Reichle (*Arch. f. Kinderhke*, 1921, LXX, 161) have introduced a micro-method based upon Hammerschlag's method for blood.

amount of total solids.¹ Normally, these range from 60 to 70 grams with a 24-hour excretion of 1,500 c.c. of urine. The urea usually constitutes about one-half of the total solids. As the normal percentage of urea is approximately two with a specific gravity of 1.020, the writer has been struck with the usual close relationship of the percentage of the urea to the specific gravity. In watching this point in over 2,000 examinations of urine within the last year, the writer has observed that the percentage of urea will practically parallel the last two figures of the specific gravity; in other words a specific gravity of 1.015, for instance, will normally be associated with a urea content of 1.5 per cent. This statement is true only in those cases which contain neither albumin nor sugar.

As a rule, it may be said that the specific gravity of the urine is inversely proportional to the amount of fluid eliminated. It will, therefore, be evident that conditions leading to an oliguria will produce a high specific gravity, while those causing polyuria will give a low specific gravity. This statement must be modified when considering certain pathologic conditions, as we may find a diminished amount of urine with low specific gravity as in chronic nephritis in which the salts are diminished, although the organic albuminous bodies are much increased; while in diabetes mellitus we have an abundant urine of high specific gravity.

The specific gravity of the urine is of more or less importance in judging of the activity of the kidneys. In acute nephritis we find a urine of high specific gravity, while in the chronic types of renal disease the specific gravity is low owing to the diminution of the salts. It is to be noted in this connection that in the case of so-called "functional" albuminuria, the specific gravity of the urine is above the normal figures. A marked reduction in the specific gravity of any case of nephritis is of dangerous import.

(7) Optical Activity.

According to Haas,² the normal urine is slightly levorotatory, ranging from 0.01 to 0.18. This optical activity is due to traces of the conjugated glycuronic acids which will be discussed later.

An increase in this levorotatory power is observed due to the presence of increased amounts of glycuronic acid, β -oxybutyric acid, albumin (in amounts over one-half part per thousand), and levulose. Dextrorotatory urines depend upon the presence of glucose, maltose, and lactose, while the presence of pentose usually gives rise to an optically inactive urine or one at least showing only a slight degree of dextrorotation.

This optical activity under the influence of pathologic products will be discussed in the section on Carbohydrates, to which the reader is referred.

¹ See Jacob, Deutsch, Arch. f. Klin. Med., 1913, CX, 1. Fischer (Ill. Med. Jour., 1921, XXXIX, 430) has introduced the term "relative concentration" by which he means the figure obtained by dividing the last two figures of the specific gravity as found by the last two figures of the normal specific gravity, which latter factor he believes to be 1022.

² Centralbl. f. d. Med. Wissensch., 1876.

III. CHEMICAL PROPERTIES

(A) Normal Composition.

The tables given in most text-books showing the chemical composition of the urine cannot be regarded as absolutely indicative of the excretion as shown in the every-day specimens of urine. The composition of the urine is absolutely dependent upon the diet under normal conditions, so that a table to be exact must embrace the findings under a specified diet.¹ Perhaps the most frequently quoted table is that of Parkes, which may be found in almost any text-book dealing with the urine. This table gives the various figures for the different substances excreted, but does not take into consideration the amount of the various types of foods used in the diet leading to this excretion. The writer, therefore, feels that it is wise to omit such a table at present, as we have no method of comparison of the excretion with the intake. The recent work of Folin upon the urine of persons both under a mixed diet and a nitrogen-free diet gives an "approximately complete" determination of the urinary constituents under absolutely fixed dietary conditions. The figures for such diets will be given under the head of each individual substance discussed.

The daily urine of a healthy adult will vary between 1,200 and 1,500 c.c. in amount and will contain from 60 to 70 grams of total solids, of which the inorganic constituents form from 25 to 30 grams and the organic between 35 and 40 grams.² The **inorganic** constituents consist of the phosphates of sodium, potassium, calcium, and magnesium, the chlorids and sulphates of the alkali metals, various types of ammonium salts, traces of nitrates,³ calcium carbonate (especially under vegetable diet), and traces of iron compounds. While these inorganic substances have not hitherto been credited with much importance, to-day we are realizing more and more that much is to be learned by a careful study of the inorganic excretion.⁴

The **organic** substances are of especial importance both in metabolic work and in the diagnosis of pathologic conditions. While we do not by any means know everything concerning the variations in excretion of these organic products, yet we do know much which is helpful in our direct diagnostic work as well as in our study of the progress of the disease. Among the organic substances which are more or less normal (although not always in large amounts) in the urine we find the lower and higher fatty acids, oxalic acid, acetone, glycerophosphoric acid, a trace of glucose, lactose (especially in nursing mothers), carbamic acid, urea, oxaluric acid, allantoin (especially a few days after birth), creatinin, uric acid, purin bases, thiosulphuric acid, taurocarbamic acid, cystin, chondroitin-sulphuric acid, inosite, hippuric acid

¹ See Long, *Jour. Am. Med. Assn.*, 1912, LVIII, 757; also, Long and Gephart, *Jour. Am. Chem. Soc.*, 1912, XXXIV, 1229. Kinloch, *Jour. Path. and Bacteriol.*, 1914, XIX, 77; Blatherwick, *Arch. Int. Med.*, 1914, XIV, 409.

² See Atkins and Wallace (*Biochem. Jour.*, 1913, VII, 219) for a discussion of the critical solution point of urine.

³ See Mitchell, Shonle, and Grindley, *Jour. Biol. Chem.*, 1916, XXIV, 461.

⁴ See Baumann and Howard (*Arch. Int. Med.*, 1912, IX, 665) for a discussion of the inorganic metabolism in scurvy.

benzoic acid, phenaceturic acid, p-oxyphenyl-acetic acid, hydro-p-cumaric acid, skatol-carbonic acid, conjugated sulphuric and glycuronic acids, oxy-proteic acid, pigments, organic iron compounds, traces of protein, and ferments.

Under pathologic conditions we may find lactic acid, large amounts of acetone, aceto-acetic acid, β -oxybutyric acid, fats, large amounts of glucose, levorotatory carbohydrates, r-arabinose, lecithin, cystin, putrescin, cadaverin, ptomaines, oxymandelic acid, leucin, tyrosin, homogentisic acid, uroleucic acid, cholesterin, cholic acid, glycocholic acid, tauro-cholic acid, various derivatives of phenol, hematin, hematoporphyrin, methemoglobin, other blood pigments, bile pigments, melanin, and protein material.

(a) Total Solids and Total Ash.

The estimation of the total solids of the urine is a matter of considerable difficulty, owing to the fact that evaporation of the urine leaves a syrupy residue which is dried only with much trouble and constant loss of ammonia, formed through the action of diacid sodium phosphate upon urea in concentrated solution. One may come very close to accurate results by placing a weighed amount of dry clean sand in a weighed platinum dish and adding 10 to 15 c.c. of urine. This is then evaporated upon the water-bath and later in the drying oven at 105°C . The dish is then placed in the desiccator and allowed to remain until the weight becomes constant. Knowing the weight of the dish and sand, the amount of the urine added, and the weight of the dish after the urine is evaporated the total solids may be easily calculated. In case a large amount of residue is desired for quantitative work, recourse may be had to the method of Slagle,¹ of adding 5 c.c. of concentrated H_2SO_4 to each liter of urine showing a specific gravity up to 1.020 and evaporating this to dryness. More H_2SO_4 must be used with the more concentrated urines.

The estimation of the ash is as follows: Fifty c.c. of urine are evaporated to dryness over the water-bath in a weighed platinum or porcelain dish. The dish is then heated, while covered, over the free flame until gases cease to be evolved, especial care being taken not to permit sputtering of the contents. In some cases it is possible completely to incinerate the urine by long-continued heat. However, a more usual procedure is to treat the carbonized residue with distilled water, thoroughly stir the mixture and filter through a filter-paper whose ash is known. The contents of the dish should be washed onto the filter several times and the material upon the filter also washed with boiling water. The filter-paper and its contents are now placed in the dish and completely incinerated. After this procedure the filtrate and washings of the original carbonized material, which contain most of the inorganic constituents, are placed in the dish and evaporated at 100°C . to dryness, and then incinerated over the free flame. The dish is now placed in a desiccator and dried to constant weight. Knowing the weight of the dish with its contents and the original weight of the dish a simple calculation will give the amount of ash in the 50 c.c. of urine taken. As originally stated the inorganic constituents will range between 25 and 30 grams under normal conditions.

¹ Jour. Biol. Chem., 1910, VIII, 77. See also, Braman, *Ibid.*, 1914, XIX, 105.

(b) Inorganic Constituents.**(1) Chlorids.**

The chlorids are one of the most important groups of inorganic solids in the urine. They are derived entirely from the food and, in consequence, the amount of excretion will depend upon the intake. The chlorids actually forming constituent parts of the food exist in combination with potassium and calcium, while those which are added as seasoning to the food are practically always in the form of sodium chlorid. As the amount contained in the food is trivial in comparison to that added, we are accustomed to regard practically all of the intake as sodium chlorid. This is a constituent of the serum of the blood and of other tissues, while the potassium salt is in more direct relation with the cellular elements.

Under normal conditions from 10 to 15 grams of sodium chlorid are eliminated in 24 hours. The administration of a diet rich in salts will increase this amount, while a salt-poor diet will diminish the amount up to a certain point. If the diet be a starvation one, or an absolutely salt-free one, the chlorids will disappear almost entirely from the urine. The regulation of the metabolic activity of the system is such that a certain amount of salt must be retained in order to preserve the osmotic equilibrium. For this reason we find that withdrawal of salt from the diet does not lead to any appreciable diminution of the normal chlorid content either of the blood or tissues. An increase in the elimination of salt is practically always followed by a retention of salt unless a sufficient supply is furnished by the food. If food containing sodium chlorid be given after a period of salt-free diet, a portion of this salt will be retained. Conversely, we find, if the body has for pathologic reasons retained sodium chlorid, that an increased elimination will follow. This metabolic activity is intimately associated with the general protein metabolism of the body. Any increase in the amount of circulating protein as compared with the living protoplasm will be followed by an increased elimination of the chlorids, which have been previously retained by the living or active protein material of the protoplasm. This fact is shown by the relation between the elimination of the chlorids and the total nitrogen. With an ordinary diet this ratio is as one to one, but in disease it may be much disturbed owing to chlorid retention through renal insufficiency. The blood and tissues of patients with nephritis show a higher chlorid content than those of normal individuals. We should, therefore, expect, if the law of increased chlorid excretion being dependent upon increased circulating albumin were to hold, that the chlorids would be increased along with the albumin in nephritis. But we find that the kidney under these circumstances is unable to excrete the increased amounts of salts circulating in the blood.

A further method of withdrawing sodium chlorid from the body consists in the administration of large quantities of alkaline carbonates or of compounds of the alkalies with vegetable acids. As soon as these are given the body becomes poor, not only in acid substances and in HCl, but also at the same time in sodium and potassium. Practically speaking, the body becomes directly impoverished in NaCl. The body may also lose chlorin when vomit-

ing is frequent, when absorption is diminished, when the stomach is regularly washed out, and when diarrhea is marked. This loss may be especially noted in cases of hyperacidity of the gastric juice associated with vomiting. The chlorin in these conditions is withdrawn in the form of free acid, and in consequence the alkalinity of the tissues may be increased.

Physiologic Variations.

The amount of sodium chlorid excreted will depend directly upon the amount ingested. We may find as high as 30 grams of salt in the 24-hour specimen or it may be as low as 2 grams, salt-free diet reducing the elimination to a mere trace. The elimination may be increased by active exercise, by increasing the water intake and hence the water output, and by the intake of a large amount of vegetable food. Much more chlorid is apparently excreted during the day than during the night.¹

Pathologic Variations.

A marked **diminution** of the chlorids, which may in some cases be almost complete, has been supposed to be pathognomonic of pneumonia. This, however, has been shown to be fallacious, as the same condition occurs in most acute febrile states, with a possible exception of intermittent fever. In a doubtful fever a large diminution in the amount of urinary chlorids might be strongly presumptive of pneumonia, but would be conclusive only in the presence of distinct clinical signs of this disease. While the retention of these chlorids in the exudate of pneumonia may partially explain the diminution in the urine, it cannot explain the fact that the chlorids of the food are also retained. The explanation is more likely to be found, in the writer's opinion, in an existing renal insufficiency.² This same retention of chlorids will be found also in any condition in which there is a transudate or exudate of any considerable bulk, so that this factor must play a certain rôle. As crisis approaches in pneumonia, the chlorids of the urine will increase in favorable cases, while in those of bad prognosis no such increase will, as a rule, be observed. Van der Bergh believes the explanation of the diminished urinary chlorids in pneumonia to be an attempt on the part of the blood to maintain its osmotic pressure, the chlorids remaining fixed in the tissues owing to the increase of the products of metabolism in the plasma.

The chlorids are diminished in all acute and chronic renal diseases associated with albuminuria. The work of Widal upon the influence of chlorids upon the progress of a renal disease has brought out the facts that not only do we have such a chlorid retention, but that the presence of chlorids in the food will increase both the albuminuria and the edema of these conditions. While these facts are incontrovertible, we must take into considera-

¹ See Borelli and Girardi, *Deutsch. Arch. f. klin. Med.*, 1914, CXVI, 216; Goldberg and Hertz, *Ibid.*, 201. Boenheim, *Ztschr. f. Exp. Med.*, 1921, XII, 295, 302 and 317.

² See Snapper, *Deutsch. Arch. f. klin. Med.*, 1913, CXI, 281; also, Hoff, *Cor.—Bl. f. schweiz. Aertze*, 1913, XLIII, 1410; Marcialis, *Policlinico*, 1920, XXVII, 86 and 184.

tion, as Richter has shown, the amount of water intake as well.¹ This is such an important field to the clinician that the writer would refer to other works giving the details of the "*dechloridization*" treatment.

A severe diarrhea will also diminish the amount of chlorids in the urine, as the chlorids of the food are carried off by the bowel too quickly to permit of absorption. In cases of carcinoma of the stomach, in dilatation either from hypersecretion or stricture of the pylorus, and in some cases of ulcer of the stomach a diminution or even total absence of chlorids in the urine may be observed.

In most chronic diseases, in anemic conditions, in rickets, and in marked nervous diseases, such as melancholia or mania, the amount of chlorids may be greatly reduced. If the output of chlorids be very low in a chronic disease, the prognosis becomes grave unless the diet can explain the diminution.²

A diminution is observed in most febrile diseases, especially in the exanthemata, while in typhoid fever the reduction is not so marked. This slight diminution in typhoid fever may serve as a distinguishing point in the diagnosis of meningitis from typhoid fever, in the former case the diminution being much more marked than in the latter. In acute yellow atrophy of the liver the chlorids are diminished, while in cirrhosis of the liver they are somewhat increased.³

The Chlorids are increased in all conditions which have previously shown a retention, according to the law which has been previously discussed. We find thus an increase in the period of convalescence from acute febrile diseases, especially pneumonia. Strangely enough, the chlorids are found markedly increased in diabetes insipidus,⁴ which is associated also with the excretion of a large amount of urine. In epilepsy an increase may be observed following the attack.

The chlorids are increased in the urine after the use of chloroform, whether administered internally or as an anesthetic.⁵ Some of the diuretics, especially potassium acetate, produce an increase in the urinary chlorids.

In metabolic work it is frequently of advantage to study the effects of an *ash-free diet* upon the pathologic condition. Taylor has,⁶ therefore, introduced such a diet, consisting of the whites of 18 eggs, 120 grams of olive oil

¹ See Fischer, Jour. Am. Med. Assn., 1915, LXIV, 325; Wilson and Hawk, Jour. Am. Chem. Soc., 1914, XXXVI, 137 and 1774; Leva, Ztschr. f. klin. Med., 1915, LXXXII, 1, Lebensohn, Jour. Biol. Chem., 1915, XXIII, 513; O'Hare, Arch. Int. Med., 1916, XVII, 711; Rackemann, Longcope and Peters, Ibid., XVIII, 406; Holt, Courtney and Fales, Am. Jour. Dis. Child., 1917, XIII, 73; Goto, Jour. Exper. Med., 1918, XXVII, 413; Zondek, Ztschr. f. klin. Med., 1919, LXXXVII, 349.

² See Barantschik, Deutsch. Arch. f. klin. Med., 1914, CXIV, 167; Landsberg, Ztschr. f. Geburtsh. u. Gynäk., 1914, LXXVI, 53; Levison, Jour. Am. Med. Assn., 1915, LXIV, 326. For a discussion of McLean's index of chlorid excretion see section on blood.

³ Burnham (Jour. Am. Med. Assn., 1912, LVIII, 851) reports a case of alcoholic cirrhosis in which there was a constant decrease in the excretion of the urinary chlorids. Guyot (Bull. Soc. Pharm. Bordeaux, 1920, LVIII, 235) reports diminution of chlorids in cases showing acetonuria.

⁴ See Förschbach and Weber, Ztschr. f. klin. Med., 1911, LXXIII, 221; also, Mever, Ibid., 1912, LXXIV, 352.

⁵ See Graham, Jour. Exper. Med., 1915, XXII, 48.

⁶ Univ. Cal. Pub., Pathology, 1904, I, 71.

and 200 grams of crystallized sugar. The work of Goodall and Joslin¹ with this diet confirms the earlier views that it is practically impossible to diminish the chlorin of the body by more than 10 to 14 per cent. and that the loss of water is proportionate to this.

Estimation of the Chlorids.

For rough clinical purposes the amount of chlorids in the urine may be estimated as follows: A few c.c. of clear, filtered urine, from which albumin if present is removed by heating with acetic acid, are placed in a test-tube and acidified with 10 drops of chemically pure nitric acid. This mixture is then treated with a few drops of 10 per cent. silver nitrate solution. If the chlorids are present in normal amount a distinct, curdy white precipitate will settle out. If the chlorids be increased a heavy precipitate will be observed, while if they be diminished only a cloud without any flakes will be seen.

Quantitative Determination.

The best method for such determination is, in the writer's opinion, the Arnold modification of Volhard's method.² The **principle** of the test is the precipitation of the chlorids in a definite amount of urine by a standard solution of silver nitrate in the presence of an excess of free nitric acid. If the precipitate of silver chlorid be filtered from the solution, the excess of silver nitrate may be determined in the filtrate by titration with a standard solution of potassium sulphocyanate, using a strong solution of iron-ammonium-alum as an indicator. The urine should be as fresh as possible and should contain no nitrites. Albumin, unless present in very large amount, need not be removed. It is wise, however, in case the urine shows a high albumin content, to acidify the urine with acetic acid, boil, and filter off the precipitated albumin. In doing this one should take a definite volume of urine, precipitate as above, and wash the precipitate thoroughly with water in order to dissolve any chlorids which may have been retained by the albumin. The filtrate is made up to a definite volume, which represents the amount of urine originally taken. Thus, 20 c.c. of urine are treated as above and washed with sufficient water to make 50 c.c. In the test as outlined later, in which 10 c.c. of urine are used, 25 c.c. of this filtered albumin-free urine will represent 10 c.c. of original urine.³

Solutions Necessary.

(1) A solution of silver nitrate of such a strength that 1 c.c. is equivalent to 0.01 gram of NaCl or 0.00606 gram of Cl. In making this solution 29.055 grams of pure anhydrous crystallized silver nitrate are dissolved in

¹ Arch. Int. Med., 1908, I, 615.

² See Bayne-Jones (Arch. Int. Med., 1913, XII, 90) for a simplification of this test; also, McLean and Selling, Jour. Am. Med. Assn., 1914, LXII, 1081; Blasucci, Ibid., 1399; Wunder, Münch. Med. Wchnschr., 1914, LXI, 2436; McLean and Van Slyke, Jour. Biol. Chem., 1915, XXI, 361; Seelman, Jour. Lab. and Clin. Med., 1916, I, 444; Alder, Ztschr. f. klin. Med., 1918, LXXXVI, 80; Halverson and Shulz, Jour. Am. Chem. Soc., 1919, XLI, 440.

³ If the urine be highly colored, it may be cleared with blood charcoal (see Larsson, Biochem. Ztschr., 1913, XLIX, 479).

1 liter of distilled water. The chemically pure AgNO_3 as found on the market is perfectly reliable and needs only an accurate chemical balance for weighing the exact amount. It is essential that this solution should contain exactly the amount specified, as the accuracy of the method depends upon the correct strength of the volumetric solutions.

(2) A solution of potassium sulphocyanate of such a strength that 20 c.c. will correspond exactly to 10 c.c. of the silver solution or, in other words, so that 2 c.c. of the cyanate solution are necessary to precipitate the silver, from each c.c. of the silver nitrate solution. Other workers use solutions of potassium sulphocyanate of somewhat different strength, but the simplicity of the calculations necessary to determine the chlorids of the urine is much increased by such a relation between the two volumetric solutions. As potassium sulphocyanate is very hygroscopic, it is impossible accurately to weight the exact amount necessary to make this solution. We, therefore, dissolve a slight excess (9 grams) of potassium sulphocyanate in approximately 1 liter of water. In order to make this solution correspond exactly to the silver solution it is necessary to find out how much water must be added to make 20 c.c. of this neutralize 10 c.c. of the silver solution. The technic is as follows: ten c.c. of the known solution of silver nitrate are measured from a buret and diluted with 50 or 60 c.c. of distilled water. Five c.c. of chemically pure nitric acid (specific gravity 1.2) and 5 c.c. of a strong solution of iron-ammonium-alum are added and thoroughly mixed. This mixture is then titrated with the potassium sulphocyanate solution whose strength is to be determined. The principle of this titration is that the KCNS first combines with the AgNO_3 , forming a white precipitate of silver sulphocyanate. At the exact point at which this combination is complete the potassium sulphocyanate will combine with the iron of the indicator forming sulphocyanate of iron which is distinctly red in color. The titration is, therefore, carried to the point at which a permanent faintly reddish-brown color appears on shaking the mixture. The number of c.c. of the sulphocyanate solution necessary to produce this end point is then read off from the buret and we are ready for our correction. As the sulphocyanate solution was intentionally made too strong, the titration should yield fewer than 20 c.c. of this solution. Supposing 18.5 c.c. of sulphocyanate solution were used we must obviously add to every 18.5 c.c. of the remaining sulphocyanate solution 1.5 c.c. of water according to the equation $C = \frac{N \times d}{n}$ in which C represents the number of c.c. of water which must be added to the remaining solution; N the total number of c.c. remaining after titration; n the number of c.c. consumed in one titration, and d the difference between the number of cubic centimeters theoretically required and that actually used in one titration.

The calculation would, therefore, run as follows:

$C = \frac{981.5 \times 1.5}{18.5} = 79.58$. We must, therefore, add to the remaining 981.5 c.c. of potassium sulphocyanate solution 79.58 c.c. of water to make the

sulphocyanate solution of such a strength that 20 c.c. will exactly precipitate the silver from 10 c.c. of the AgNO_3 solution.

(3). A cold saturated solution of iron-ammonium-alum. This must be absolutely chlorin free.

(4). Chemically pure nitric acid, chlorin-free and having practically no trace of nitrous acid. Specific gravity 1.2.

Technic.

Ten c.c. of urine or 25 c.c. of the diluted urine from which the albumin has been removed are accurately measured with a pipet and placed in a 100 c.c. volumetric flask. Five c.c. of nitric acid, 50 c.c. of water, and 20 c.c. of the standard silver solution are then added and the mixture thoroughly shaken. After this mixture has stood for about 10 minutes distilled water is added up to the graduating mark of the flask, after which the whole is thoroughly mixed and the precipitated silver chlorid allowed to settle. This mixture is then filtered through a perfectly dry filter into a thoroughly dry 50 c.c. volumetric flask. This 50 c.c. of filtrate will represent, therefore, only 5 c.c. of urine, but the calculation made later will compensate for this.

This 50 c.c. is then poured into a beaker of about 250 c.c. capacity and the volumetric flask is thoroughly washed out with water, the washings being added to the solution in the beaker. Five c.c. of the alum solution are then added and the mixture titrated with the potassium sulphocyanate solution to the appearance of the first permanent reddish tinge of the solution. The number of c.c. of sulphocyanate solution, necessary to neutralize the excess of silver remaining after the chlorid of silver has been filtered off, is then read off from the buret.¹

Calculation.

As 20 c.c. of the sulphocyanate solution are equivalent to 10 c.c. of the silver solution, it is evident that the number of c.c. of silver solution not used in the precipitation of the chlorids corresponds to the number of c.c. of sulphocyanate solution necessary to neutralize the 50 c.c. of the filtrate. We, therefore, subtract the number of c.c. of sulphocyanate solution used from 20 (the number of c.c. of silver solution added) and obtain directly the number of c.c. of silver solution necessary to precipitate the chlorids in 10 c.c. of urine. As each c.c. of silver solution represents 0.01 gram of NaCl or 0.00606 gram of Cl , multiply these factors by the number of c.c. used, the percentage of chlorids in the urine being obtained by multiplying the amount of chlorids in 10 c.c. by 10, and the total amount by simply multiplying this figure by the number of hundreds of c.c. in the total 24-hour specimen of urine.

If the urine is very highly colored it is advisable to add a few drops of a con-

¹ Atkinson (Jour. Lab. and Clin. Med., 1920, VI, 160) advises the use of a composite reagent containing all the elements of the above test with the exception of the thiocyanate, which is made of such a titer that 90 c.c. of the reagents equals 20 c.c. of the standard thiocyanate employed. Bell and Doisy (Jour. Biol. Chem., 1921, XLV, 427) have introduced a method for chlorids in tissues consisting of ashing the tissues with dilute HNO_3 and H_2SO_4 and distilling the HCl formed into a standard AgNO_3 solution. The excess of the silver is then titrated by Volhard's method. See, also, Rusznyak, Biochem. Ztschr., 1921, CXIV, 23.

centrated solution of potassium permanganate before the titration. This will usually decolorize the urine so that the end point will be much more distinct.

Purdy's Centrifugal Method.

This method, while having nothing in common with the accuracy of the preceding one, is very convenient and has the advantage of yielding quick results which are clinically available.¹

Ten c.c. of clear, filtered, albumin-free urine are placed in a centrifuge tube which is graduated to 15 c.c. One c.c. of strong nitric acid and 4 c.c. of a 5 per cent. solution of silver nitrate are then added. The tube is shaken by inversion and the mixture allowed to stand for a few minutes, after which it is placed in the centrifuge and whirled for three minutes at the rate of 1,200 revolutions per minute. The bulk percentage of silver chlorid is then read off, from which the percentage by weight both of sodium chlorid and of chlorin, equivalent to the precipitated silver chlorid, may be calculated. One per cent. by bulk represents 0.13 per cent. by weight of NaCl and 0.08 per cent. of Cl.

As previously stated, the amount of chlorin in the urine depends upon the amount ingested, ranging normally between 10 and 15 grams. By the use of Folin's standard diet, which contains 6.2 grams of Cl, the excretion is found to be 6.1 grams of Cl in 24 hours. On the ash-free diet of Taylor the excretion at the end of 12 days of such a diet was 0.17 gram of Cl in the 24 hours.

(2) Phosphates.

The phosphates occurring in the urine are the sodium, potassium, calcium, and magnesium salts of the tribasic orthophosphoric acid (H_3PO_4). As previously stated, in the discussion on the reaction of the urine, normal, clear, acid urine contains no dibasic monacid phosphates, but all of the phosphates under these conditions are of the monobasic diacid type. If the urine becomes less acid or amphoteric in reaction we find, however, in addition to the above, the disodium monohydrogen phosphate, the monocalcium phosphate, and the monomagnesium phosphate; while if the urine be alkaline we may find the neutral phosphates in the ascendency. It must be remembered, therefore, that the normal acidity of the urine is not strictly regulated by variations in the relative proportions of the monosodium dihydrogen phosphate and of the disodium monohydrogen phosphate as usually stated. Besides these mineral phosphates, phosphoric acid is found in the urine in combination with glycerin as glycero-phosphoric acid, which is derived largely from the hydrolytic cleavage of lecithin compounds. According to Mandel and Oertel, Mathison,² and Yoshimoto,³ the output of organically bound phosphorus is not increased by a phosphorus-rich diet, while Kondo⁴ shows that, although absolutely increased in amount, its relation to total P_2O_5 is diminished.

¹ See Sucyoshi, Mitt. a. d. med. Fak. der k. Univ., Tokyo, 1916, XIV, 425; Agasse-Lafont, Douris and Hayem, Bull. de l'Acad. de Med. Paris, 1916, LXXVI, 521; Achard, *Ibid.*, 572.

² Biochem. Jour., 1909, IV, 274.

³ Ztschr. f. physiol. Chem., 1910, LXIV, 464.

⁴ Biochem. Ztschr., 1910, XXVIII, 200. See, also, Plimmer, Biochem. Jour., 1913, VII, 43.

The larger portion of the urinary phosphoric acid is derived from the food, the smaller portion coming from the metabolism of the tissue protein, especially the nucleins. This endogenous phosphoric acid may be of special importance as variations will be found depending upon the degree of destruction of the lecithin and nuclein compounds. It is to be remembered here that not all of the phosphoric acid ingested is excreted, as between a fourth and a third of the total quantity may remain in the feces in combination with calcium. In studying the effects of increased ingestion of phosphates, the feces must, therefore, be examined quite as closely as the urine.

Physiologic Excretion.

The amount of phosphoric acid excreted in the 24 hours is always expressed in terms of P_2O_5 . The normal P_2O_5 excretion of the adult varies from 1 to 5 grams with an average of about 3.5 grams. The figures of Folin, based upon a diet containing 5.9 grams of P_2O_5 , show this excretion to average 3.87 grams in 24 hours, while patients on an ash-free diet eliminate approximately 0.75 gram. In this excretion the phosphates of sodium and potassium usually exceed those of calcium and magnesium, the former being excreted in the amounts of 2 to 4 grams in the 24 hours, the latter from 1 to 1.5 grams. Little data exists regarding pathologic variations in the relation of these two types of phosphates so that no conclusion may at present be drawn. The excretion of P_2O_5 will vary with the food, especially with the amount of calcium and magnesium of the food. These bases combine in the intestine with the phosphoric radical forming phosphates which are difficultly soluble.¹ This fact is taken advantage of by Croftan in the administration of calcium salts to precipitate the phosphates and thus diminish their activity in conditions attributable to uric acid.

The phosphates are increased on an animal diet and diminished on a vegetable diet as Zülzer has shown. During starvation an increase of the phosphates may be observed, as an indication of decomposition of the tissues. Administration of phosphates at this time will usually lead to a retention to counterbalance the previous loss. This same fact was observed in the discussion of the chlorids and may be stated as a general law, that an increased excretion is followed by a retention and a retention by an increased elimination. It must be stated, however, that an insufficient supply of phosphoric acid is not compensated for by such a great retention of phosphates as of the chlorids. The organism eliminates even more phosphoric acid in starvation than in cases of deprivation of salt, as the decomposing protein sets free the salts bound up with it. Many attempts have been made to determine where and in what form phosphoric acid is retained in the body and where and from what sources the body draws upon it for excretion. It is a difficult matter to determine what amount of the phosphoric acid retained reaches the bones, what portion is devoted to the soft tissues, and how much of it remains organically combined in the body (Magnus-Levy). It has been

¹ See Würtz, *Biochem. Ztschr.*, 1912, XLVI, 103; also, Knox and Tracy, *Am. Jour. Dis. Child.*, 1914, VII, 409. Telfer (*Biochem. Jour.*, 1921, XV, 347) has shown that the degree to which the normal excretion of calcium and phosphorus can be varied depends on the concentration of free fatty acids in the intestinal contents.

found that the relation between the excretion of phosphoric acid and nitrogen is normally about one to seven, the same relations which exist between the amount of nitrogen and phosphoric acid in the human muscular tissue. It is, therefore, plausible to assume that a retention of both nitrogen and phosphoric acid will lead to a deposition of increased flesh. In starvation we find that this relation is markedly disturbed, the phosphoric acid being both relatively and absolutely increased. Such being the case the loss of P_2O_5 must be largely sustained by the bones, which are relatively poor in nitrogen. For a full discussion of this subject the writer would refer to von Noorden's work on Metabolism and Practical Medicine, which gives great detail regarding all phases of metabolism.

The phosphates are increased during hard muscular exercise, while mental exercise seems to lead to a diminished excretion of the alkaline phosphates and an increased output of the earthy phosphates. The ingestion of large quantities of water is frequently associated with an increased elimination of the phosphates, although this is later followed by a slight retention.

It not infrequently happens that a freshly voided urine shows a marked turbidity and even precipitation due to the deposit of earthy phosphates. This has been supposed to be due to an increased output of the phosphates, but it is now known to be nothing but the natural consequence of a change of urinary reaction from acid to alkaline. This condition which has been called "*phosphaturia*" would, therefore, much more appropriately be styled "*alkalinuria*." This subject will be discussed in a later section to which the reader is referred.¹

Pathologic Variations.

A **diminished elimination** may be observed in cases of acute febrile disease, especially at the height of pneumonia. The degree of diminution is usually proportionate to the severity of the disease and usually lessens as convalescence comes on. According to Gouraud, the earthy phosphates are considerably reduced in pneumonia, while in tuberculous conditions the phosphates are increased, an interesting point in differential diagnosis. This retention in pneumonia as well as in the other acute febrile diseases is possibly due to the renal insufficiency which may be very great in such conditions. This diminished phosphatic excretion may not always obtain in the acute febrile conditions, in some cases a sudden increased output being observed. In typhoid fever Robin believes an increased elimination during the febrile rise to be an unfavorable sign, while an increase during defervescence indicates a favorable prognosis.

The phosphates appear to be diminished in most chronic diseases. In all renal diseases, whether acute or chronic, a diminished excretion is present due to the renal insufficiency. Failure of the kidneys to excrete acid phosphates may lead to a type of acidosis, as the renal elimination of phosphates represents one of the most important normal mechanisms for maintaining the neutrality of the blood.² This diminished phosphoric acid

¹ See Dünner, Deutsch. Med. Wchnschr., 1915, XLI, 973; Fiske, Jour. Biol. Chem., 1921, XLIX, 171.

² See Greenwald, Jour. Biol. Chem., 1915, XXIX, 21; Marriott and Howland, Arch. Int. Med., 1916, XVIII, 708; Denis and Minot, Ibid., 1920, XXVI, 99.

excretion is regarded by Purdy¹ as a factor almost as constant as is the excretion of albumin. In gout the phosphoric acid excretion runs parallel to that of uric acid, decreasing immediately preceding the acute attack and rising as the attack subsides. In cases of pregnancy a diminished excretion is observed which is attributable to the withdrawal of phosphoric acid from the maternal organism for the purpose of the fetal bone formation. In certain bone diseases, such as osteomalacia, a diminished excretion is usually observed, although at times an actual increase is seen. The earthy phosphates, especially, are diminished in these latter conditions while the alkaline phosphates may be increased. In cases of myositis ossificans the excretion of inorganic phosphates does not seem to be much affected as one might expect from the new bone formation.

In cases of hystero-epilepsy the phosphates are diminished, the diminution usually being proportionate to the intensity of the attack, while in true epilepsy the phosphates appear to be more or less markedly increased. It is in just the nervous diseases that one would expect to find much variation in the phosphatic excretion, but very few data are found bearing on this subject. Folin and Shaffer find that in the periods of nervous excitement the relative amount of phosphoric acid is diminished, but that the absolute amount is little changed.

In Addison's disease, hepatic cirrhosis, acute yellow atrophy, and chronic lead-poisoning we may find an extensive decrease of phosphates in the urine.

In certain cases which show most of the symptoms of diabetes mellitus without any sugar output, a **phosphatic increase** is observed in the urine. This condition has been called "*phosphatic diabetes*," and may be associated with the excretion of as high as 10 grams of P_2O_5 within 24 hours. In true diabetes mellitus the phosphates may be increased at one time and diminished at another, as there seems to be an inverse ratio between the excretion of sugar and that of the phosphates.

The phosphates seem to be increased in cases of pseudoleukemia, leukemia, hemorrhagic purpura, in cases of acute or chronic inflammatory processes of the genito-urinary tract, and in cyclic vomiting of children.

As previously stated, the output of urinary nitrogen bears a relation of about seven to one to that of the phosphate excretion. This relation has been termed the "*relative value*" of phosphoric acid and represents the amount of P_2O_5 corresponding to 100 grams of N. Normally this ranges between 15 and 20

Estimation of Phosphates.

Ten c.c. of urine are rendered alkaline with ammonia. The earthy phosphates are precipitated in the form of a flocculent precipitate and may be roughly estimated by the volume of the precipitate.

If this alkalinized urine be filtered and the filtrate acidified with acetic acid, the addition of a few drops of ferric chlorid or of uranium nitrate solution will precipitate the alkaline phosphates.

These methods are purely qualitative and can have no clinical value

¹ Practical Urinalysis, Phila., 1900, p. 56. See, also, Marriott and Howland, Arch. Int. Med., 1916, XVIII, 708.

beyond giving a general idea of the relative amounts of the earthy and alkaline phosphates. Instead of ferric chlorid or uranium solution, magnesium mixture may be used for this purpose.

Quantitative Determination.

The usual method for the estimation of the urinary phosphates is that of titration with uranium nitrate or acetate solution. The *principle* of this method is that phosphoric acid compounds in acetic acid solution give, on treatment with uranium nitrate, a yellowish-white flocculent insoluble precipitate of uranium phosphate (UO_2HPO_4). As a means of recognizing the point at which an excess of uranium solution is present in the titrated fluid, one may use either a solution of ferrocyanid of potassium which gives a distinct brownish color at the end point, or, preferably, a few drops of tincture of cochineal, which gives a grass-green color and has the advantage that it can be added directly to the titrated fluid, which is not the case with the ferrocyanid of potassium.¹

Necessary Solutions.

(1) A solution of uranium nitrate or acetate of such a strength that 20 c.c. shall correspond to 0.1 gram of P_2O_5 . It is a matter of absolute indifference whether the acetate or the nitrate be used, but the writer prefers the nitrate as this is more easily obtained in the pure state. In making up a solution of uranium nitrate of the above strength, one may not rely implicitly on the weighing, as the uranium nitrate may contain impurities or excess water and thus vitiate the results. It is, therefore, necessary to have a standard phosphate solution against which the uranium solution may be titrated.

The usual solution recommended by various writers is one of disodium monohydrogen phosphate. This salt varies in its degree of hydration and its solutions do not keep well. Moreover, it is absolutely necessary when this salt be used that a definite amount of it be taken and converted into sodium pyrophosphate, after which a corresponding dilution of the solution must be made to make it of such a titer that every 50 c.c. shall be equivalent to 0.1 gram of P_2O_5 . In view of these facts, the writer is accustomed to follow the suggestion of Giles² and use chemically pure dihydrogen monopotassium phosphate. This salt crystallizes well without any water of crystallization and does not alter on exposure to the air.

This solution is to be made such a strength that 50 c.c. corresponds to 0.1 gram of P_2O_5 , in other words a liter must contain 2 grams of P_2O_5 . In order to find out just how much of this salt must be dissolved in a liter of water we must have recourse to a simple calculation. The formula of dihydrogen monopotassium phosphate is KH_2PO_4 , its molecular weight being 136. Two molecules of this salt are necessary to yield one molecule of P_2O_5 according to the equation



If, therefore, one liter of the solution must contain 2 grams of P_2O_5 , the

¹ See Sato, Jour. Biol. Chem., 1918, XXXV, 473, for a micro method based on this test.

² Sutton's Volumetric Analysis, Philadelphia, 1904, p. 294.

amount of KH_2PO_4 which must be dissolved in a liter is easily calculated from the following proportion:

$$272 : 142 :: x : 2. \quad x = 3.83$$

We, therefore, dissolve 3.83 grams of dihydrogen monopotassium phosphate in 1 liter of water and obtain directly a solution which contains 2 grams of P_2O_5 or one in which every 50 c.c. is equivalent to 0.1 gram of P_2O_5 . It is perhaps, needless to add that this solution should be made in an accurately standardized volumetric flask, and at the temperature at which the flask is calibrated.



FIG. 71.—Volumetric flasks.

Having thus obtained our standard phosphate solution, we are now in a position to make up our standard uranium nitrate solution, the titer of which must be such that 20 c.c. corresponds to 0.1 gram of P_2O_5 , or, in other words, one liter of which must be equivalent to 5 grams of P_2O_5 . The formula of uranium nitrate is $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, its molecular weight being 502.6. Uranium nitrate combines with dihydrogen potassium phosphate according to the following equation:



As seen above, two molecules of the dihydrogen phosphate are necessary to yield one molecule of P_2O_5 . We will, therefore, have, when uranium nitrate acts upon the dihydrogen phosphate, only the equivalent of $\frac{1}{2}$ molecule of P_2O_5 ; that is, 71 parts. As the uranium solution must contain 5 grams of P_2O_5 to the liter we may then calculate how much uranium nitrate is necessary to form the equivalent of such a solution by the following proportion:

$$502.6 : 71 :: x : 5. \quad x = 35.39.$$

Were we absolutely certain of the purity and state of hydration of our uranium nitrate, all that would be necessary would be to weigh out this

exact amount. As this is not the case, we weigh out a slight excess (35.75 grams) and dissolve in one liter of distilled water. We are now ready to determine the strength of the uranium solution as follows:

Fifty c.c. of the dihydrogen monopotassium phosphate solution are placed in a beaker and treated with a few drops of tincture of cochineal and 5 c.c. of acetic acid mixture (see below, solution 2). Some workers prefer the addition of potassium ferrocyanid as an indicator, but this does not give as distinct a contrast at the end point, and if tests are made by adding a drop of the mixture to the ferrocyanid solution on a white plate, loss of substance must occur. This mixture is then heated and titrated, as soon as the boiling point is reached, with the uranium solution until a trace of a distinct green color becomes permanent on stirring the mixture. Duplicate determinations are then made, the results of which should agree exactly with the original. The number of c.c. of uranium solution used is then read off and we are prepared for the calculation of the amount of water which must be added to standardize the solution.

As 20 c.c. of this uranium solution should correspond exactly to 50 c.c. of the standard phosphate solution, we may, for the sake of example, use the same figures given for obtaining the dilution in the case of the sulphocyanate solution discussed under the heading of Chlorids. Thus if 18.5 c.c. of uranium solution were used we must add, according to the previous explanation, 79.58 c.c. of distilled water to the remaining 981.5 c.c. of uranium solution in order to make every 20 c.c. equivalent to 0.1 gram of P_2O_5 .

(2) An acetic acid mixture prepared by dissolving 100 grams of sodium acetate and 30 grams of glacial acetic acid in sufficient water to make 1000 c.c. This solution must be added in the determination of the urinary phosphates in order to overcome the influence of the nitric acid liberated in the reaction and to convert any monacid phosphates into the diacid type.

(3) An indicator, preferably tincture of cochineal prepared by digesting the ground cochineal bugs in 25 per cent. alcohol and filtering. This indicator has the advantage that it may be added directly to the solution to be titrated, while potassium ferrocyanid must be used by the plate method of adding a few drops of the solution to the indicator after each addition of uranium solution.¹

Technic.

Fifty c.c. of clear filtered urine are placed in an Erlenmeyer flask and treated with 5 c.c. of the acetic acid mixture, for the purpose of transforming any monacid phosphates into the diacid form and of neutralizing the nitric acid formed during the titration. A few drops (5 to 10) of tincture of cochineal are added, the mixture heated to the boiling-point, and then titrated as described above. It is wise invariably to run duplicate determinations. After each addition of the uranium nitrate the precipitate is allowed to settle so that one may see more clearly the first trace of any green coloration or precipitate.

¹ Mitchell (Hahnemann Monthly, 1921, LVI, 152) shows that ammonium carbonate, which is present in some urines, affects the ferrocyanid indicator to a marked degree and may lead to fallacious results.

The calculation is as follows: Supposing 10 c.c. of the uranium solution were used, the corresponding amount of P_2O_5 in the 50 c.c. of urine examined would then be found from the equation:

$$20 : 0.1 :: 10 : x. \quad x = 0.05.$$

The percentage of P_2O_5 would, therefore, be 0.1 (2×0.05). If the total 24-hour urine were 1,500 c.c., the total P_2O_5 excretion would be obviously 1.5 grams.

Method of Bell and Doisy.¹

Inorganic Phosphates.

One to five c.c. of urine, according to concentration, are accurately measured into a 100 c.c. volumetric flask and 25 c.c. of phosphate-free water added. In a similar flask are placed 5 c.c. of the standard phosphate solution,² also with 25 c.c. of water. To both flasks are added 5 c.c. of the molybdic acid solution³ and 5 c.c. of the hydroquinone solution.⁴ After standing 5 minutes, 25 c.c. of the carbonate-sulphite solution⁵ are added and the flasks made up to volume and mixed. While the color of the mixtures is not absolutely permanent, yet there is no appreciable change within an hour. However, as the solutions in the cups of the colorimeter do not fade at the same rate as that in the flasks, both cups of the colorimeter should be filled at the same time. The comparisons are made in the Duboscq or other colorimeter in the usual way. If the standard solution be set at 20 m.m., the calculation is as follows:

$$\frac{10}{\text{Reading } X \text{ c.c. urine used}} = \text{Gm. inorganic P per liter.}$$

Total Phosphorus.

One c.c. of urine is measured with an Ostwald pipet into a hard glass test-tube and 6 to 8 drops of concentrated H_2SO_4 , 1 c.c. of concentrated HNO_3 , and a piece of quartz are added. The tube is cautiously heated until nitrous fumes no longer come off and the remaining drops of H_2SO_4 are colorless. Care should be taken to avoid evaporating to dryness or pronounced overheating which may cause loss of phosphoric acid. The remaining few drops

¹ Jour. Biol. Chem., 1920, XLIV, 55.

² Pure mono-potassium phosphate is finely ground and exposed in a desiccator over H_2SO_4 for several days. Of this dry preparation 4.394 grams are dissolved and made up to 1 liter with phosphate-free water in an accurate volumetric flask. One c.c. of this solution contains 1 mg. of phosphorus. The solution should be preserved with chloroform and kept tightly stoppered. Of this stock solution, 50 c.c. are accurately measured into a 500 c.c. volumetric flask and made up to volume with phosphate-free water. This solution should, also, be preserved with chloroform. If it becomes turbid or should molds appear, a new solution should be prepared. Five c.c. of this solution contain 0.5 mg. of phosphorus.

³ Fifty grams of pure ammonium molybdate are dissolved without heat in 1 liter of phosphate-free normal sulphuric acid. Five c.c. of this solution, treated with an equal amount of the hydroquinone solution and, after standing 5 minutes, with 25 c.c. of the carbonate-sulphite solution, should give an absolutely colorless mixture.

⁴ Twenty grams of pure hydroquinone are dissolved in 1 liter of phosphate-free water and 1 c.c. of concentrated H_2SO_4 is added. On standing, this solution becomes colored due to the formation of some quinone. A moderate amount of color does no harm as the quinone is reduced in the determination by the alkaline sulphite solution. The solution should be kept tightly stoppered to reduce the oxidation by the air.

⁵ To 2 liters of a 20 per cent. solution of Na_2CO_3 are added 75 grams of sodium sulphite dissolved in 500 c.c. of water. The mixture is then filtered.

of H_2SO_4 are transferred to a 100 c.c. volumetric flask with about 25 c.c. of phosphate-free water. Into a similar volumetric flask, containing 4 to 6 drops of concentrated H_2SO_4 , are measured 2, 3, or 5 c.c. of the standard phosphate solution mentioned in the test for inorganic phosphorus, the amount to be added being determined by the result found for the inorganic phosphate. Having been brought to the same volume with phosphate-free water, the standard solution and the urine residue are treated and compared as in the determination of inorganic phosphate. If the standard be set at 20 m.m., the calculation is as follows:

$$\frac{2 \text{ X c.c. standard used}}{\text{Reading}} = \text{Gm. total P per liter.}$$

Organic Phosphorus.

While it might be considered possible to obtain the amount of organic phosphorus by subtracting the inorganic phosphorus from the total phosphorus yet, as pointed out by Taylor and Miller,¹ this is not permissible as the amount of organic phosphorus is so small. The normal output of this type of phosphorus varies from 0.01 to 0.1 gram in 24 hours. Phosphorus-rich diets increase the absolute amount but diminish the relative percentage.

Twenty c.c. of urine are measured into a 25 c.c. volumetric flask, made just alkaline with powdered barium hydroxid, filled to the mark with water, and filtered. The precipitate contains barium sulphate and phosphate. To remove the excess of barium, 20 c.c. of this filtrate are measured into another 25 c.c. flask, made just acid with dilute H_2SO_4 , filled to volume, and filtered. 10 c.c. of this last filtrate (equivalent to 6.4 c.c. of urine) are evaporated with 8 to 10 drops of concentrated H_2SO_4 to about 2 c.c., and 2 c.c. of concentrated HNO_3 are added. The digestion is carried out as given under "Total Phosphorus" and the residue transferred to a 25 c.c. flask with 10 c.c. distilled water. In another similar flask are placed 5 c.c. of the diluted standard solution² (which is used for estimation of phosphates in blood), 4 to 6 drops of concentrated H_2SO_4 , and 5 c.c. of water. To both flasks are added 1 c.c. of the molybdic acid solution and 2 c.c. of the hydroquinone (both given under "Inorganic Phosphates" above). After 5 minutes, 10 c.c. of the carbonate-sulphite solution are added, and the flasks made up to volume and the comparisons made as usual, the standard being preferably set at 30 or 40 m.m. The calculation is as follows; if the standard is at 40:

$$\frac{1000}{\text{Reading X } 6.4} = \text{Mg. organic P per liter.}$$

Purdy's Centrifugal Method.

This method cannot be relied upon for accurate results in metabolic work, but may of be some service from the clinical standpoint. Ten c.c. of clear filtered urine are placed in a centrifuge tube graduated to 15 c.c.

¹ Jour. Biol. Chem., 1914, XVIII, 215.

² Five c.c. of the stock phosphate solution, given in foot-note 2 under Inorganic Phosphates above, are made up to 1000 c.c. and preserved with chloroform. Five c.c. of this solution contain 0.025 mg. of phosphorus.

Two c.c. of 50 per cent. acetic acid, and 3 c.c. of 5 per cent. uranium nitrate solution are then added and thoroughly mixed with the urine by inversion of the tube. The tube is then placed in the centrifuge and operated at a speed of 1200 revolutions for three minutes. According to Purdy, 1 per cent. by bulk of uranium phosphate equals 0.04 gram of P_2O_5 in each 100 c.c. of urine. Each succeeding percentage by bulk increases by the figure 0.01. Thus a bulk percentage of five of uranium phosphate would equal 0.04 plus 0.04, or 0.08 gram of P_2O_5 in each 100 c.c. These figures are much at variance with those of Ogden, who states that "he has found that each $\frac{1}{10}$ of a c.c. of precipitate calculated as P_2O_5 is equivalent to 0.0225 per cent. by weight." Owing to these differences, the writer would suggest that the bulk percentage be stated as such rather than as parts by weight of P_2O_5 .

(3) Sulphur Compounds.

The sulphur is present in the urine in three forms: (1) preformed or neutral sulphates; (2) ethereal or conjugated sulphates, sulphuric acid in combination with aromatic compounds, and (3) neutral, unoxidized, or organic sulphur. The total output of sulphur depends essentially upon the protein metabolism, both of that of the tissues and of the food.¹ It is to be remembered that the sulphur elimination is much less accurate than that of the nitrogen as an indication of the degree of protein metabolism, owing to the fact that different protein substances vary in their sulphur-content. The daily excretion of sulphur, in terms of SO_3 , varies from 1 to 3.5 grams, when the subject is upon a mixed diet. Ordinarily, the ethereal sulphates form about one-tenth of the total output. The neutral sulphur does not vary under normal conditions as far as its absolute amount is concerned, but we note, on changing the diet to one which is relatively free in protein material, that the relative amount of the neutral sulphur is markedly increased. Thus, Folin finds on a diet containing 18.9 grams of nitrogen and 3.8 grams of SO_3 the daily excretion of total SO_3 is 3.31 grams, of which the inorganic SO_3 is 2.92 (87.8 per cent. of total), the ethereal SO_3 is 0.22 (6.8 per cent.), and the neutral SO_3 0.17 (5.1 per cent.). On a nitrogen-free diet, consisting of cream and arrowroot, a total SO_3 excretion of 1.04 grams is noted, of which 0.63 gram (60.6 per cent.) is traceable to the inorganic SO_3 , 0.12 (11.5 per cent.) to the ethereal SO_3 , and 0.29 (27.9 per cent.) to the neutral SO_3 . We are, therefore, confronted with the following fact, "the distribution of the sulphur in urine among the three chief normal representatives, inorganic sulphates, ethereal sulphates, and 'neutral sulphur,' depends on the absolute amount of sulphur present."

On the ash-free diet of Taylor we find, according to Goodall and Joslin, a total SO_3 secretion of 0.96 gram, of which the inorganic SO_3 forms 0.71 gram (74 per cent.), the ethereal sulphates 0.05 gram (5.2 per cent.), and the neutral sulphur 0.2 gram (20.8 per cent.).

It is doubtless true that practically all of the urinary sulphur is derived from protein metabolism, a definite relation being usually established between the nitrogenous and sulphur output. Normally, $N : SO_3 :: 5 : 1$, apparently regardless of whether the patient is on a nitrogen-rich or a nitro-

¹ See Cathcart and Green, *Biochem. Jour.*, 1913, VII, 1.

gen-free diet; the absolute amounts of each, however, differ markedly, depending upon the diet. Folin's figures show that as the total urinary sulphur is reduced, the percentage represented by the inorganic sulphates sinks from about 90 to 60 per cent. This fact has been expressed in the above quotation from Folin.

The reduction in the inorganic sulphates must be made up by a relative increase in the other forms of sulphur. The **ethereal sulphates** have for a long time been held to be an accurate index of the degree of absorption of the products of intestinal protein decomposition. There can be no question but that increased intestinal decomposition is associated with increased output of the conjugated sulphuric acids, especially the indoxyl and skatoxyl sulphuric acids. These ethereal sulphates are diminished on a milk diet or on the cream and arrowroot diet of Folin or the vegetarian diet adopted by many of Chittenden's subjects. This is true especially as regards the urinary indican which is absolutely negative in such cases, while the total amount of ethereal sulphate is diminished only about 50 per cent. Such being the case we must have some other than intestinal origin for the large relative increase of the ethereal sulphates under a nitrogen-free diet. I quote from Folin:¹ "(1) The urinary indican is not to any extent a product of the general protein metabolism, is therefore probably, as is generally supposed, a product of intestinal putrefaction, and may consequently be assumed to indicate approximately the degree of putrefaction in the intestinal tract. (2) The ethereal sulphates can only in part be due to intestinal putrefaction, and neither their absolute nor their relative amount can be accepted as an index of the extent to which the putrefaction is taking place in the intestines. (3) The ethereal sulphates, on the contrary, represent a form of sulphur metabolism which becomes more prominent when the food contains little or no protein." Here the sulphuric acid is conjugated with aromatic bodies formed from decomposition of tissue protein.

Pathologic Variations.

The sulphates, as a whole, must be increased in any condition associated with increased protein catabolism. Thus we find in febrile conditions an increased output of sulphur corresponding to the intensity of the process, this increase being followed by a diminution as convalescence comes on. An increased elimination of sulphates has been observed in leukemia, diabetes mellitus and insipidus, progressive muscular atrophy, and following the use of such drugs as morphin, potassium bromid, sodium salicylate and acetanilid. From the clinical standpoint the elimination of sulphates has little practical value, the variation in the amounts of ethereal sulphates and neutral sulphur being the chief factors of value.²

While, as shown above, the **ethereal sulphates** are subject to great variation, the indoxyl-potassium sulphate (indican) varying according to the degree of intestinal decomposition, we find some points of clinical interest in their study. As the putrefactive processes normally occur below the ileo-cecal

¹ *Loc. cit.*

² See Stadtmüller and Rosenbloom, *Arch. Int. Med.*, 1913, XII, 276; Ross, *Ibid.*, 1914, XIII, 889; Greenwald, *Ibid.*, 1914, XIV, 374.

valve, any condition increasing such decomposition with a consequent increase of urinary indican would indicate trouble in the lower bowel, more frequently of the chronic type. They are increased in cases showing abnormal intestinal absorption, as, for instance, in typhoid fever, intestinal tuberculosis, peritonitis, and chronic intestinal catarrh. Obstructive jaundice is usually associated with increase in the output of indican as the bile seems to have a great influence upon putrefactive processes in the intestine. They appear to be increased in cholera, while in ordinary diarrhea they are diminished in absolute amount, but may be relatively increased. In cases of gastric hypoacidity associated with bacterial decomposition in the stomach the urine may show a marked increase of indican. In acute nephritis a very intense indican reaction may be obtained, while in the chronic form the amount of indican is usually diminished. In cases of pus-formation almost anywhere within the system an increased elimination of ethereal sulphates may be observed due to the absorption of the products of decomposition of the pus. This point is of some importance in differentiating purulent from non-purulent affections of various organs. The writer has seen a persistent intense indicanuria, associated with middle-ear infection, which cleared up completely after thorough drainage.

Neutral Sulphur.

The neutral sulphur does not have a very definite relation to the amount of sulphur of the intake or to the amount formed in the decomposition of tissue protein. While it is said to vary in amounts representing from 12 to 15 per cent. of the total SO_3 of the urine, this must be true only when the absolute amount of excreted SO_3 is taken into consideration. By this is meant that although the absolute amount of neutral sulphur does not normally vary to any great extent, its percentage relation to the total sulphur varies with the amount of sulphur. Thus Folin finds on an intake of 3.8 grams of SO_3 an output of 0.17 gram of neutral SO_3 , while on a nitrogen-free diet the output is 0.2.

The nature of the neutral sulphur of the urine is somewhat uncertain, as we seem to have only two well-established bodies, namely, the sulphocyanates and hydrogen sulphid. The sulphocyanates are derived largely from the absorption of material from the saliva and represent approximately one-third of the total neutral sulphur. The hydrogen sulphid may be regarded as a decomposition product. Besides these we find cystein, which is an intermediary product of normal protein metabolism, and tauro-carbamic acid, which is derived from the biliary material. In cases of jaundice we may find as high as 60 per cent. of the sulphur in the neutral form, which may be due to the absorption of this and other biliary material. Traces of chondroitin-sulphuric acid, oxyproteic acid, alloxypoteic acid, and uroferic acid contribute to the neutral sulphur-content of the urine.

The greatest increase of neutral sulphur is probably associated with the presence of cystin which is not normally present in the urine. This is undoubtedly derived from abnormal protein decomposition. The sulphur in the neutral form may reach as high as 30 or 40 per cent. of the total, due

to the presence of cystin. The writer will refer to a later section for a discussion of the subject of cystinuria.

The variations in the neutral sulphur of the urine must be regarded as indicative of abnormal metabolic processes which are not associated with variations in the other types of urinary sulphur. As Folin has shown, "the neutral sulphur is not at all due to processes identical or similar to those which give rise to indican. The neutral sulphur represents products which in the main are independent of the total amount of sulphur eliminated or of protein catabolized."

Estimation of Total Sulphur (A).

The method followed by the writer in determining the total urinary sulphur in terms of SO_3 is the Folin¹ modification of the Asboth-Modrakowsky method. Twenty-five c.c. of urine (50 c.c. if very dilute) are measured into a large nickel crucible of about 200 c.c. capacity and treated with 3 grams of sodium peroxid.² The mixture is evaporated to a syrupy consistency and is then carefully heated to dryness. The latter part of this procedure requires about 15 minutes. The crucible is removed from the flame and allowed to cool. Moisten the residue with 1 or 2 c.c. of water, add about 7 grams of sodium peroxid and heat the mixture to complete fusion for about 10 minutes. Allow the fused mass to cool, add about 100 c.c. of water and heat to boiling for at least one-half hour to dissolve the alkali and decompose the peroxid. This mixture is transferred to an Erlenmeyer flask (about 400 c.c. capacity) by means of hot water and is diluted to about 250 c.c. Concentrated HCl is slowly added to the almost boiling solution until the oxid of nickel (formed in the process) just dissolves. This will require about 18 c.c. of acid for 8 grams of Na_2O_2 . After boiling for a few minutes, the solution should be perfectly clear. If it is not clear too much water or too little peroxid were used in the last fusion. Allow the mixture to cool and filter from the insoluble residue, if there is any. To this clear acid solution add 5 c.c. of very dilute (1 to 4) alcohol and boil for a few minutes to remove the last traces of chlorin, which are formed on acidifying the solution. Now add, drop by drop, 10 c.c. of a 10 per cent. barium chlorid solution and allow the precipitate of BaSO_4 to settle out for *two days* in the cold. At the end of this time the precipitate is filtered off, using either ash-free filters, or preferably, weighed porcelain Gooch crucibles with properly prepared asbestos mats. Wash the precipitate with about 250 c.c. of cold water, dry and ignite for 15 minutes. In this heating of the Gooch crucible, the flame must touch neither its perforated bottom nor its sides. If the crucible be placed upright upon the cover of a platinum crucible, which rests upon a platinum triangle the flame may be applied directly to the bottom of the platinum. After the heating, the crucible is placed in the desiccator, is allowed to cool and is weighed. Knowing the

¹ Jour. Biol. Chem., 1906, I, 131; Jour. Amer. Chem. Soc., 1908, XXXI, 284. See, also Raiziss and Dubin, Jour. Biol. Chem., 1914, XVIII, 297; Fiske, *Ibid.*, 1921, XLVII, 50; Robison, Biochem. Jour., 1922, XVI, 134.

² Benedict, Denis, and Schmidt use, as the oxidizing agent, a solution of copper nitrate and potassium chlorate. See Tsuji, Biochem. Jour., 1915, IX, 439; Drummond, *Ibid.*, 492; Givens, Jour. Biol. Chem., 1917, XXIX, 15.

weight of the crucible and mat before and after this process, a simple calculation yields the amount of BaSO_4 obtained from the urine taken. If the filter-paper be used, the precipitate is washed as previously mentioned. Transfer the filter-paper and precipitate directly to a weighed platinum or porcelain crucible, add 3 or 4 c.c. of alcohol and ignite, to dry and partially burn the filter-paper. Heat the residue until complete incineration occurs and the ash becomes colorless. Cool the crucible in a desiccator and weigh. If the amount of BaSO_4 obtained by either of these methods be multiplied by 0.3429, the result is the amount of SO_3 in the urine taken. A simple calculation yields the percentage and total amount excreted.

Determination of Total Sulphates (B).

Folin's Methods.

Under this head we determine the neutral (preformed or inorganic) and etherealsulphates. Folin¹ has advanced two methods, the first of which is preferable.

1. *Precipitation in the Cold*.—Twenty-five c.c. of urine and 20 c.c. of dilute hydrochloric acid (1 to 4), or 50 c.c. of urine and 4 c.c. of concentrated HCl , are gently boiled in an Erlenmeyer flask (capacity about 200 c.c.) for 20 to 30 minutes (never less than 20). To prevent loss of material it is wise to keep the flask covered with a small watch-glass during the boiling. The flask is cooled for two or three minutes in running water and the contents are then diluted with cold water to about 150 c.c. To this solution are then added 10 c.c. of a 5 per cent. barium chlorid solution, care being taken not to shake or stir the contents during the addition. At the end of an hour or more, the mixture is shaken up and filtered. The rest of the determination is as outlined above.

2. *Precipitation in the Heat*.—The boiling of the urine with hydrochloric acid is conducted exactly as in the preceding method. At the end of 20 to 30 minutes, the boiling urine is diluted to about 150 c.c. with hot water. The mixture is heated once more to the boiling point, is removed from the flame, and immediately precipitated by the addition of 5 c.c. of 10 per cent. barium chlorid solution, which must be added drop by drop. The mixture is allowed to stand for 2 hours in order to cool. The remainder of the process is as previously given.

Determination of Inorganic (Neutral) Sulphates (C).

About 100 c.c. of water (not less), 10 c.c. of dilute hydrochloric acid (1 to 4), and 25 c.c. of urine (50 c.c. if dilute, when a correspondingly smaller amount of water is taken) are measured into an Erlenmeyer flask of about 200 c.c. capacity. 10 c.c. of a 5 per cent. solution of barium chlorid are added, using the drop method, the urine solution not being shaken or disturbed during this addition. At the end of an hour or more, the mixture is shaken and filtered. The remaining steps are given above. The figure obtained here represents the amount of SO_3 referable to the preformed sulphates.

The ethereal sulphates (D) need not be determined separately as the difference between the total sulphates (B) and the inorganic sulphates (C) will represent the amount of these ethereal sulphates in the specimen ex-

¹*Loc. cit.* See, also, Rosenheim, *Biochem. Jour.*, 1914, VIII, 143.

aminated. If the SO_3 referable to the total sulphates (B) be subtracted from the SO_3 of the total sulphur (A) the remainder represents the neutral sulphur¹ in terms of SO_3 .

Purdy's Centrifugal Method.

Ten c.c. of clear urine are placed in a centrifuge tube and to it are added 5 c.c. of barium chlorid mixture, consisting of four parts of barium chlorid, one part of concentrated hydrochloric acid, and 16 parts of distilled water. The tube is inverted to insure mixing of the reagent and urine and is allowed to stand for a few minutes. It is then placed in a centrifuge and whirled three minutes at the rate of 1,200 revolutions per minute. Each percentage of BaSO_4 by bulk represents approximately 0.25 per cent. of SO_3 by weight. This result can, of course, not be as accurate as the preceding, but has some clinical advantage.

(4) Carbonates.

A freshly voided specimen of urine may contain small quantities of carbonates and bicarbonates and some free carbonic acid.² The amount of free carbonic acid varies with the degree of acidity of the urine and the amount of carbonate-forming material in the food. It has been found that vegetable foods are almost always productive of an alkaline urine, owing to the fact that the organic acids of the vegetables may be converted into carbonates and excreted as such. The carbonate which most frequently forms as a sediment in the urine is calcium carbonate which will be treated in a later section.

If the urine be acidified and a stream of air passed through this acidified urine into a vessel containing a solution of barium hydrate, the carbon-dioxid liberated by the acids will unite with the barium forming barium carbonate which may be filtered, dried, and weighed. Mitchell³ has introduced the following simple test for the detection of alkaline carbonates in urine. Fill a small test-tube half full of the urine and, by means of a small pipet, add to it about 5 drops of a 1 per cent. aqueous mercurous nitrate solution, in such a manner that the solution forms an upper stratum on the urine. If alkaline carbonates (or sulphids) be present, a dark precipitate will immediately be seen in the upper stratum of urine. If they are absent or only in traces, a white precipitate appears. If, now 50 per cent. acetic acid (10 drops or more) be added, the dark precipitate turns to a brown or gray and immediately settles if the original dark color was due to carbonates, while if due to sulphids the dark precipitate is not affected.

(5) Sodium and Potassium.

These metals exist in the urine in the form of the oxids Na_2O and K_2O , the former being present in amounts of 4 to 7.5 grams, the latter varying

¹ See Liebesny, *Biochem. Ztschr.*, 1920, CV, 43.

² Denis and Minot, *Jour. Biol. Chem.*, 1918, XXXIV, 569, report the total CO_2 output in 24 hours as varying from 20 to 211 c.c. Based upon a series of measurements of P_H and of the concentrations of free and bound carbonic acid, Gamble (*Jour. Biol. Chem.*, 1922, LI, 205) concludes that free carbonic acid in urine is a nearly stationary value. The bicarbonate content of urine varies inversely with the urinary P_H . The reaction of voided urine may rapidly increase in alkalinity because of loss of free CO_2 unless collected with precaution.

³ *Medical Rec.*, 1921, XCIX, 516.

from 2 to 4 grams. The normal relation between the excretion of these products is as 5 to 3.

The sodium is largely derived from the addition of sodium chlorid to the diet, while the potassium is a constituent of most vegetable foods. We find, therefore, that both of these substances depend largely upon the diet in normal cases, while in pathologic conditions the potassium may be excreted in larger amounts.¹ Thus we find in fever the potassium salts predominate over the sodium compounds up to the time of crisis, after which the sodium salts again assume their normal proportions. Increased exercise as well as increased decomposition of protein from pathologic causes will tend to increase the potassium of the urine. From the clinical standpoint the quantitative determination of the sodium and potassium of the urine has little significance.²

(6) Calcium and Magnesium.

Both of these alkali-earth metals are excreted in the urine largely in the form of the phosphates. Calculated as the oxids, the calcium excretion (CaO) varies from 0.1 to 0.3 gram and the magnesium (MgO) from 0.15 to 0.4 gram in 24 hours, the relation between the calcium and magnesium excretion being about 1 to 1.5, although Nelson and Burns have shown that any given individual may exhibit a constant behavior as to whether calcium or magnesium shall be greater in the urine voided.

The chief source of these compounds is the food, but it must be remembered that only a small portion of these substances is excreted in the urine. Calcium and, to a less extent, magnesium form compounds with phosphoric acid in the bowel and are excreted without being absorbed. Even if these compounds be injected subcutaneously the excretion is largely into the intestine. We should, therefore, study much more closely the calcium metabolism by examination of both the feces and urine.

Little is known regarding the output of either of these metals, but what work has been done is more closely connected with the calcium excretion than that of magnesium. During starvation calcium oxid is increased both relatively and absolutely, its probable source being the bones. It is increased to some extent by exercise and is diminished after the administration of alkalis. In chronic diseases we note an increase due, probably, to inanition. In tubercular conditions the calcium output is occasionally found to be diminished, while some cases of a marked increase have been noted. In diabetes mellitus, as in other conditions associated with an acidosis, the output of CaO may be greatly increased, thus running parallel to the ammonia output. In a case of myositis ossificans studied by the writer there seemed to be no great variation in the calcium output, while the magnesium content of the urine was markedly reduced. Austin has reported similar findings. McCrudden and Fales report (*Arch. Int. Med.*, 1912, IX, 273) a loss of calcium and a retention of magnesium in non-puerperal osteomalacia. The same authors (*Jour. Exper. Med.*, 1912, XV, 450) find the calcium excretion in the

¹ See Blumenfeldt, *Inaug. Dissert.*, Berlin, 1912; also, *Ztschr. f. exp. Path. u. Therap.* 1913, XII, 523; Hamburger, *Biochem. Ztschr.*, 1915, LXXI, 415.

² See Tisdall and Kramer, *Jour. Biol. Chem.*, 1921, XLVIII, 1.

urine, in cases of intestinal infantilism, to be almost negligible. Peabody (Jour. Exper. Med., 1913, XVII, 71) shows a retention of calcium and a normal excretion of magnesium in pneumonia.¹

Quantitative Determination.

Calcium.

McCrudden² has recently introduced a method for this determination which, in my opinion, yields the best results. If the urine be alkaline it must be made neutral or slightly acid with HCl and be filtered. If turbid or faintly acid to litmus, add 10 drops of concentrated HCl. Two hundred c.c. of the clear acid urine are treated with 10 c.c. of a 2.5 per cent. oxalic acid solution and 8 c.c. of 20 per cent. sodium acetate solution. Allow the mixture to stand over night at room temperature or shake vigorously for 10 minutes. Filter off the precipitate of calcium oxalate and wash it Cl-free with 0.5 per cent. ammonium oxalate solution. Save the filtrate for the determination of magnesium. The precipitate of calcium oxalate is then dried on the filter-paper at 100°C. and is placed in a weighed platinum crucible and burned over the Bunsen flame until the filter-paper is completely ashed. The blast-lamp is then applied and the crucible and contents blasted for 15 or 20 minutes. The crucible is dried in the desiccator and weighed, the increase in weight representing the calcium oxid (CaO) in the 200 c.c. of urine. A simple calculation will yield the amount of CaO in the 24-hour specimen.

Magnesium.

The filtrate and washings obtained in the above determination are treated with one-third the volume of 25 per cent. ammonium hydrate. A precipitate of magnesium ammonium phosphate (NH_4MgPO_4) occurs, which is allowed to settle for several hours, is collected on an ash-free filter, is thoroughly washed with water containing one-third its volume of ammonia, and is dried in the oven at 100°C. The filter-paper and precipitate are placed in a weighed platinum crucible and burned until the filter-paper is completely destroyed, after which the crucible and contents are heated over the blast-lamp for 15 minutes. This heating converts the magnesium ammonium

¹ See, also, Voorhoeve, Deutsch. Arch. f. klin. Med., 1913, CXI, 29. For various studies of the metabolism of calcium see Diller and Rosenbloom, Am. Jour. Med. Sc., 1914, CXLVIII, 65; Bergeim, Stewart and Hawk, Jour. Exper. Med., 1914, XX, 218 and 225; Bookman, Am. Jour. Dis. Child., 1914, VII, 436; Rosenbloom and Cohn, Arch. Int. Med., 1914, XIV, 203; Kleinschmidt, Berl. klin. Wchnschr., 1915, LII, 29; Da Costa, Funk. Bergeim and Hawk, Pub. from Jefferson Med. Coll. and Hosp., 1915, VI, 1; Bookman and Epstein, Am. Jour. Med. Sc., 1916, CLI, 267; Blatherwick, Ibid., 432; Rosenbloom, Ibid., CLII, 256; Vance, Ibid., 693; Kahn and Kahn, Arch. Int. Med., 1916, XVIII, 212; Nelson and Williams, Jour. Biol. Chem., 1916, XXVIII, 231; Nelson and Burns, Ibid., 237; Givens and Mendel, Ibid., 1917, XXXI, 421, 435 and 441; Stehle, Ibid., 461; Sawyer, Baumann and Stevens, Ibid., 1918, XXXIII, 103; Givens, Ibid., XXXIV, 119; Sherman, Gillett and Pope, Ibid., 373; Givens, Ibid., XXXV, 241; Goto, Ibid., XXXVI, 355; Kriebel and Bergeim, Ibid., 1919, XXXVII, 179; Underhill, Honeij, and Bogert, Proc. Nat. Acad. Sc., 1920, VI, 79; Holt, Courtney and Fales, Am. Jour. Dis. Child., 1920, XIX, 97; Eppinger and Ullmann, Wiener Arch. f. inn. Med., 1920, I, 639; Sherman, Jour. Biol. Chem., 1920, XLIV, 21; Mason, Ibid., 1921, XLVII, 3; Blühdorn, Ztschr. f. Kinderl. u. Jugendheilk., 1921, XXIX, 43; Hasselmann, Münch. med. Wchnschr., 1921, LXVIII, 1080.

² Jour. Biol. Chem., 1910, VII, 83; Ibid., 1911, X, 187. See, also, Lyman, Ibid., 1915, XXI, 551; Mayer, Presse med., 1917, XXV, 61; Shohl and Pedley (Jour. Biol. Chem., 1922 I, 537) have modified McCruddens method to permit of more rapid work. See, also, Shohl, Ibid., 527.

phosphate (NH_4MgPO_4) into magnesium pyrophosphate ($\text{Mg}_2\text{P}_2\text{O}_7$); one part of magnesium pyrophosphate represents 0.36243 part of MgO . All that is necessary, therefore, is to multiply the amount of magnesium pyrophosphate by the above factor to obtain the amount of MgO in 200 c.c. of urine.

(7) Iron.

Iron is practically always present in the urine in organic combination. The amount actually present is very small, being given by Magnier as varying between 3 and 11 mg. to the liter, while Neumann and Mayer find the normal output to average 0.983 mg. The urinary iron has little clinical significance. It is increased in fever, in malaria, diabetes mellitus, and pernicious anemia, the amounts in some cases running as high as 20 mg. during the 24 hours.

Any method for the estimation of the urinary iron must be very delicate and very accurate. The urine must be completely incinerated, the method of Neumann¹ employing a mixture of concentrated sulphuric and nitric acid being by far the best.²

(c) Organic Constituents.

(1) Nitrogenous Bodies.

(a) Total Nitrogen.

From the metabolic standpoint the estimation of the total nitrogen of the urine is one of the most important features of its chemical examination. The excretion of total nitrogen varies with the amount of nitrogen of the food and with the degree of tissue metabolism. Normally, the system so adapts itself to the nitrogenous intake that the excretion of nitrogen, in the urine, feces, perspiration, etc., is equal to the intake. In other words, a normal person is in *nitrogenous equilibrium*. This subject of nitrogenous equilibrium is of such great importance and the factors which influence it so varied that the writer feels that it will be unwise to discuss it briefly for fear of not presenting it clearly to the student's mind. As a full discussion of this subject would be too extensive for the scope of this work the writer must be content with reference to the admirable discussion by Magnus-Levy³ in von Noorden's Hand-book of Pathology of Metabolism.

The total nitrogen of the urine may be taken as a direct index of the protein metabolism. Upon a starvation diet, or one from which the nitrogenous factors have been eliminated, we find a gradual reduction in the amount of urinary nitrogen. From about the fourth day of starvation the excretion becomes practically constant and continues until severe tissue-decomposition, as a sign of impending death, occurs. If, at this time, a diet rich in nitrogen be given, a certain amount of the intake will be retained, but not all. If this increased nitrogenous diet be continued a certain portion will be retained each day until the system again assumes a condition of nitrogenous equilibrium, the output equaling the intake. It is to be remembered in this connec-

¹ Arch. f. Anat. u. Physiol., Physiol. Abth., 1900, S. 159.

² See Goodman (Jour. Biol. Chem., 1912, XII, 37) for the urinary iron in pneumonia; Lichtenstein, Acta Pediat., 1921, I, 194; Spiro, Schweiz. med. Wchnschr., 1921, LI, 580; Kisch, Wiener Arch. f. inn. Med., 1921, III, 283.

³ Chicago, 1908.

tion that the carbohydrates and fats of the diet both have a certain direct influence in diminishing the protein metabolism.

The normal amount of total nitrogen of the urine, with the subject upon a mixed diet, varies between 10 and 16 grams per day. The work of Chittenden¹ has proven that this is much too high for individuals who desire to get the most out of their system with the least possible work. In other words "physiological economy" is much better subserved by a diet yielding from 5 to 6 grams of total nitrogen. The subjects of his experiments showed normal activities, both mental and physical, and at the end of the experiments felt much better than before them and had gained in weight. Folin² in his work has shown that the normal excretion of six subjects, each observed for a period of five days, was 16 grams of total nitrogen on a diet of 119 grams of protein yielding 18.9 grams of nitrogen. On a nitrogen-free diet the excretion averaged 3.6 grams. He says: "It may, therefore, be positively stated, as a principle in the chemistry of metabolism, that the distribution of the nitrogen in urine among urea and the other nitrogenous constituents depends on the absolute amount of total nitrogen present."

The subject of the distribution of the various nitrogenous products of the urine has been much changed by this work of Folin and of Chittenden. This distribution or, as it is better called, the "nitrogen partition of the urine," varies according to the diet. The following table shows the excretion of the various nitrogenous constituents and their percentage relations to the total nitrogen under a mixed diet and under one which is nitrogen free.

	Excretion in grams.		Percentage of total N.	
	Mixed Diet.	N-Free Diet.	Mixed Diet.	N-Free Diet.
Nitrogen,	16.00	3.60	100.00	100.0
Urea N,	13.90	2.20	86.87	61.7
Ammonia N,	0.70	0.42	4.37	11.3
Creatinin N,	0.58	0.60	3.63	17.2
Uric Acid N,	0.12	0.09	0.75	2.5
Undetermined N,	0.70	0.29	4.37	7.3

It will thus be seen that the total nitrogen excretion of the urine is made up of several factors. The principal points to be gained from the above table are that the urea, on a nitrogen-free diet, is markedly reduced. This reduction must naturally be made up by increase in the other factors. We find the ammonia and especially creatinin markedly increased from the percentage standpoint, although both are absolutely diminished. We have, therefore, a distinctly endogenous nitrogen metabolism as well as an exogenous one. The urea content should, consequently, be considered as of direct importance in estimating the degree of protein tissue metabolism, although not as usually

¹ Physiological Economy in Nutrition, New York, 1905; Mendel, Jour. Am. Med. Assn., 1914, LXIII, 819; Benedict, Jour. Biol. Chem., 1915, XX, 263; Pepper and Austin, *Ibid.*, 1915, XXII, 81; Osborne and Mendel, *Ibid.*, 241; Johnston and Veeder, Am. Jour. Dis. Child., 1917, XIII, 404; Richet, C. R. soc. de biol. de Paris, 1918, LXXXI, 133; Robinson, Biochem. Jour., 1922, XVI, 131.

² *Loc. cit.*

taught. This urea output cannot at the present time be considered as representing from 85 to 90 per cent. of the total endogenous nitrogen, but should be regarded more properly as representing between 60 and 65 per cent. It is true that the urea as found under mixed diets gives this higher figure, but at least 20 per cent. of this must be placed against useless activity on the part of the system. In other words, the intake of sufficient nitrogen to yield a urea excretion amounting to 85 per cent. of the total N must be considered unnecessary. This is a somewhat enlarged expression of the fact that most people eat much more than is utilized by the system.¹

It is seen, therefore, that in the general run of urine examinations the urea output does not represent to us the extent of the tissue metabolism, as ordinarily we have not sufficiently controlled the diet. The above table presents a remarkable percentage increase in the amount of creatinin. The figures of Folin show that the absolute quantity of creatinin eliminated, whether upon a nitrogen-rich or on a nitrogen-free diet, is remarkably constant for the same individual. Although this is influenced to a slight extent by the diet, it is so slight that it can be disregarded (see Creatinin).

Physiologic Variations.

An increase in the total nitrogen is observed after a heavy protein meal. As previously stated, in starvation the nitrogen of the urine becomes constant after about the fourth day. This nitrogen excretion observed in starvation is, however, less than the minimum amount that must be given in the form of protein in order to maintain a nitrogenous equilibrium. As a rule, the amount of protein taken in is much in excess of the requirement of the system, so that the amount excreted probably represents protein which has never become a part of the system. For this reason the fallacy of considering urea as a direct representative of the protein metabolism of the tissues becomes evident. The intake of protein is, in reality, readjusted to suit the actual needs of the body, so that the urea can represent only a portion, about 60 per cent., of the total metabolic activity of the tissue protein. A physiological increase in the excretion of nitrogen is observed in the infant for four or five days after birth. The nitrogen excretion is increased when the intake of water has been greater than normal. This fact should be borne in mind in metabolic experiments in which the intake of water should be quite as much regulated and as well known as the intake of other substances.

Physiologically, a diminished output of nitrogen is observed on a low nitrogen diet and also on a diet rich in carbohydrates and fat, as these latter substances provide the greater part of the necessary energy.² The system must then utilize its own protein. Increased exercise is supposed to give a slight increase in the nitrogen output owing to increased muscular activity, but it is to be said that the loss of water through the increased perspiration may be such a factor in diminishing the urinary nitrogen that no increase

¹ See Mendel and Lewis, *Jour. Biol. Chem.*, 1913, XVI, 19, 37 and 55.

² McElroy and Pollock (*Jour. Biol. Chem.*, 1921, XLVI, 475) show that the rate of nitrogen elimination is an index of the rate of digestion and absorption.

may be observed. Certain medicaments as quinin and opium will usually diminish the output of nitrogen.

Pathologic Variations.

Increased Excretion.

Perhaps the most marked increase in the urinary nitrogen is observed in the acute febrile infections.¹ This increase is not due to the temperature *per se* nor is there any parallelism between the urinary nitrogen and the degree of temperature. Whether or not this increase in nitrogen can be directly traced to the effects of the toxins produced by the organisms causing the disease is still unsettled. That this cannot be the only element is proven by the fact that Krehl and Matthes have shown that more protein is destroyed in the so-called aseptic fever than is the case in the normal organism under similar conditions, dietetic and physical. In some febrile conditions we find that the elimination of nitrogen may be reduced during the febrile period, while about the time of crisis a very marked output of urinary nitrogen may be observed. This is the well-known epicritical elimination of nitrogen. In the fever associated with acute nephritis the urinary nitrogen is not increased, but is rather diminished owing to the renal insufficiency as well as to the edema which occurs. A toxogenic decomposition of protein is found in cases of carcinoma, pernicious anemia, chronic tuberculosis, leukemia, scurvy, and especially in exophthalmic goiter. In cases of acute yellow atrophy and phosphorous poisoning the total nitrogen may be increased, but the percentage of urea will be very much diminished.

In cases of diabetes mellitus the nitrogen excretion is usually much increased, due more to the effect of the increased nitrogenous diet than to increased endogenous protein metabolism. Likewise, in diabetes insipidus a large increase in urinary nitrogen may be observed. An increase is occasionally observed in cases of nephritis owing to the large albumin content of the urine. During the progress of absorption of an exudate a very high excretion may be observed, the resolution of a pneumonic exudate, for instance, being easily followed by the variations in the urinary nitrogen.²

Diminished Excretion.

A diminution in the nitrogen excretion is usually observed in convalescence from acute and chronic conditions. This is probably due to the attempt on the part of the system to make up for the losses incurred during the active progress of the disease. It may be diminished in conditions in which the absorptive power of the intestine is much reduced. If the oxidative powers of the system are very much reduced as the result of chronic conditions, the urinary nitrogen will usually be much diminished. In cases of nephritis, both acute and chronic, a large diminution in the urinary nitrogen is observed. This is due primarily to the renal insufficiency and to the associated dropsy. In such conditions a marked increase may be observed in the fecal nitrogen,

¹ See Sharpe and Simon, *Jour. Exper. Med.*, 1914, XX, 282.

² See Matthes and Miller, *Jour. Biol. Chem.*, 1913, XV, 87; also, Gammeltoft, *Habilitationschr.*, Copenhagen, 1913.

especially when marked diarrhea is a complicating factor. These periods of retention of nitrogen in nephritis may alternate with periods of increased elimination, so that examinations at different periods may show greatly conflicting results. When the water output of the urine is largely diminished, as a result of transudation, of exudation, or of increased perspiration, the total nitrogen may be reduced.¹

Estimation of Total Nitrogen (Kjeldahl).

The principle of this method is as follows:² The nitrogenous constituents of the urine are oxidized by various oxidizing agents into ammonia. This ammonia is converted into ammonium sulphate by the sulphuric acid which is added at the same time as the oxidizing agents. After the preliminary decomposition and oxidation of the organic nitrogen into ammonium sulphate, free ammonia may be liberated by the action of strong sodium hydrate and distilled into a standard acid solution. Knowing the strength of the acid solution, one may then titrate the remaining acid with a standard alkali solution and determine how much ammonia has combined with the acid. One c.c. of tenth-normal sulphuric acid, used by the ammonia liberated in the distillation, represents 0.001401 gram of nitrogen.

Technic.

Five c.c. of urine are accurately measured, either with a pipet or buret, into a Kjeldahl flask of Jena glass of 800 c.c. capacity. Ten c.c. of concentrated sulphuric acid³ and approximately 1 gram of copper sulphate are added, the flask placed in a hood and heated over a low flame until white fumes of sulphuric acid are given off (Gunning's modification). Five grams of potassium sulphate⁴ are then added and the mixture heated with an increased flame to boiling for one-half to three-fourths of an hour. The solution should have lost every trace of a yellowish color and should have become by this time a clear bluish-green. The worker should be cautioned regarding the fumes given off in this process and conduct his work only in a hood with a good draft. It is frequently necessary to wash down the carbon from the sides of the vessel by shaking the fluid in such a way that the carbonized material is carried down to the bottom of the flask. One should be cautious lest he lose some of the liquid in this manipulation, which would not only throw out his determination but might result in a very severe burn should any of the material fall upon him.

¹ Minot, Bull. Johns Hopkins Hosp., 1914, XXV, 332; Hefter and Siebeck, *Deutsche Arch. f. klin. Med.*, 1914, CXIV, 497; Frothingham and Smillie, *Arch. Int. Med.*, 1915, XV, 204; Murray, *Brit. Med. Jour.*, 1915, I, 151; Abderhalden, *Ztschr. f. physiol. Chem.*, 1915, XXVI, 1; Mosenthal and Richards, *Arch. Int. Med.*, 1916, XVII, 320; Wilson, Bull. Johns Hopkins Hosp., 1916, XXVII, 121; Foster and Davis, *Am. Jour. Med. Sc.*, 1916, CLI, 49; Losee and Van Slyke, *Ibid.*, 1917, CLIII, 94; Jeans, *Am. Jour. Dis. Child.*, 1917, XIII, 145; Schamberg and Raiziss, *Jour. Cut. Dis.*, 1917, XXXV, 135; Lewis, *Jour. Biol. Chem.*, 1917, XXXI, 363. Sullivan, Stanton and Dawson (*Arch. Int. Med.*, 1921, XXVII, 387) and Sullivan (*Ibid.*, 1921, XXVIII, 119) show that the total N is decreased in pellagra.

² See Folin and Farmer (*Jour. Biol. Chem.*, 1912, XI, 493) for a micro-chemical method based on this Kjeldahl process; Gradwohl and Blaivas, *Jour. A. M. A.*, 1916, LXVII, 809.

³ Faaron (Dublin *Jour. Med. Sc.* 1920, 4th Ser., 28) advises a mixture of sulphuric and phosphoric acids.

⁴ See Scott and Meyers, *Jour. Am. Chem. Soc.*, 1917, XXXIX, 1044.

The mixture is allowed to cool completely before the further steps of the determination can be taken. Most workers advise at this juncture the transference of the material from the first flask into a second distilling flask. The writer has convinced himself that this procedure is not only unnecessary but is even unwise, as the transference may result in slight loss of material. He is, therefore, accustomed to use the same flask both for the oxidation and distillation.

After the mixture has cooled the neck of the flask is thoroughly washed with a stream of distilled water so that every trace of material may be carried from the neck into the body of the flask. Sufficient additional water is added to bring the total up to approximately 250 c.c. A little talcum powder, a few pieces of pumice stone, or a few pieces of granulated zinc may then be added

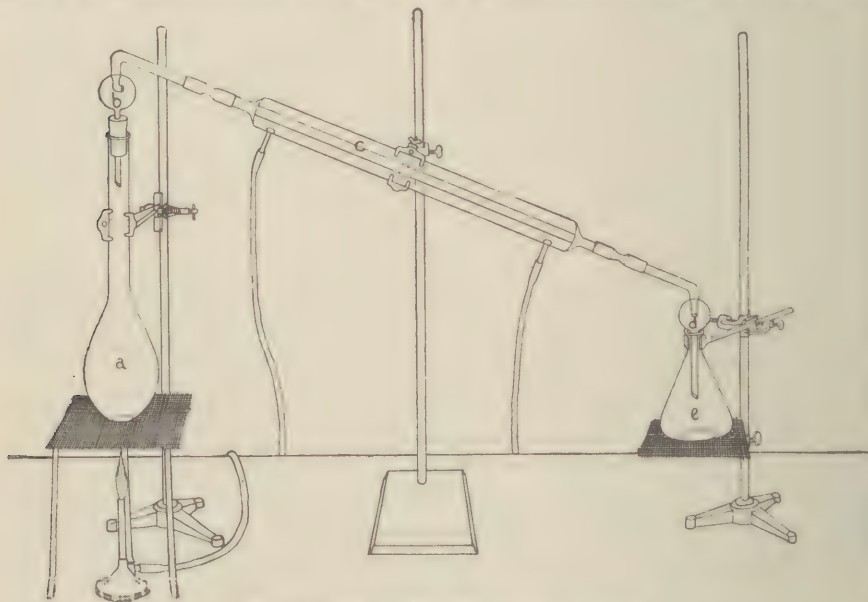


FIG. 72.—Kjeldahl's nitrogen apparatus.

to prevent bumping of the contents when sodium sulphate separates out later in the process. Fifty c.c. of 40 per cent. sodium hydrate are then added for every 10 c.c. of sulphuric acid used in the original oxidation. Care should be taken in adding this strong alkali that none of it touches the upper portion of the neck of the flask. The alkalized mixture is then shaken and connected with a Fresenius bulb which is attached to a Liebig condenser as shown in the accompanying cut. The outlet tube passes into an Erlenmeyer flask which should contain 50 c.c. of tenth-normal sulphuric acid. In cases with abnormally high nitrogen values it may be necessary to use a larger quantity than 50 c.c. of standard acid, but the writer has found only two instances in over 1,500 determinations in which an increased amount was necessary. The connection of the distilling flask to the bulb and condenser should be done rapidly to avoid any possible loss of ammonia.

The distilling flask is heated slowly at first and the heat increased only

after boiling has become regular. If heated too quickly, spurting of the liquid may occur and traces of alkali be carried into the bulb and thence over into the standard acid. The distillation should continue until about 150 c.c. have been distilled over, which will take from 20 to 30 minutes.

The writer has observed that bumping of the mixture rarely occurs, under the conditions outlined above, before the ammonia is completely driven over. This can, however, not be taken as an absolute sign that every trace of ammonia has been distilled off. In order to see whether such is the case, one must test the outlet tube with a piece of moist red litmus-paper which will turn blue in the presence of traces of ammonia. If all ammonia has not been given off, the distillation must be continued until such is the case. If no more ammonia is being evolved, the distilling flask is disconnected from the bulb so that no suction may draw the standard acid into the condenser. The connecting bulb is removed and the tube of the condenser washed with a spray of distilled water so that any material adhering may be washed into the standard acid. The outlet tube is disconnected and washed both internally and externally into the standard acid solution.¹

The standard acid solution is then titrated with tenth-normal sodium hydrate solution, using cochineal, methyl-orange, alizarin-red, or methyl-red as an indicator. The writer prefers the use of the latter. As each c.c. of tenth-normal alkali is equivalent to each c.c. of tenth-normal acid, we subtract, from the original number of c.c. of acid (50), the number of c.c. of standard alkali used to neutralize the remaining standard acid. The difference gives us the number of c.c. of standard acid neutralized by the ammonia given off in the distillation. As each c.c. of tenth-normal acid is equivalent to 0.001401 gram of nitrogen, we multiply this factor by the number of c.c. of acid neutralized by the ammonia and obtain the amount of total nitrogen in 5 c.c. of urine. This result multiplied by 20 yields, of course, the percentage of total nitrogen, which may be changed into the actual total amount of nitrogen by multiplying it by the number of hundreds of c.c. in the 24-hour specimen. It goes without saying, that the reagents used in this determination must be ammonia-free or, at least, that their ammonia content be known.

Folin and Wright's Simplified Kjeldahl Method.

This method² has the advantages that it requires very little equipment and that the determination can be finished easily in 20 to 25 minutes. The hydrolyzing-oxidizing reagent used consists of the following mixture: 50 c.c. of 5 to 6 per cent. copper sulphate solution, 300 c.c. of "syrupy" phosphoric acid (about 85 per cent. strength), and 100 c.c. of concentrated sulphuric acid.

Technic.

Transfer 5 c.c. of undiluted urine to a 300 c.c. Kjeldahl flask, preferably a Pyrex flask. Add 5 c.c. of the above digestion mixture, 2 c.c. of 10 per cent. ferric chlorid solution, and 4 to 6 small pebbles or pieces of granite to

¹ See Dakin and Dudley, *Jour. Biol. Chem.*, 1914, XVII, 275; Citron, *Deutsch. med. Wchnschr.*, 1920, XLVI, 655; Reyner, *Jour. Lab. and Clin. Med.*, 1921, VII, 53; Mestrezat and Janet, *C. R. Acad. des Sc.*, 1920, CLXXI, 1019.

² *Jour. Biol. Chem.*, 1919, XXXVIII, 461.

prevent bumping. Boil this mixture vigorously in a hood, using a micro-burner. In 3 to 4 minutes the foam which forms at first will entirely disappear and the flask becomes filled with dense white fumes. When this stage is reached (but no earlier) cover the mouth of the flask with a small watch-glass and continue the vigorous heating for 2 minutes. At the end of this time, dilute urines will already be green or blue and concentrated ones will be a light straw-yellow, the carbonaceous matter being completely destroyed. The flame should then be turned low and the gentle boiling process should be continued for 2 minutes, making a total boiling period of 4 minutes counting from the time the watch-glass was put in place. Remove the flame and let the flask cool for 4 to 5 minutes. At the end of 4, or not more than 5, minutes, add first 50 c.c. of water, then 15 c.c. of saturated sodium hydrate (50 to 55 per cent.) and connect the Kjeldahl flask promptly, by means of a rubber stopper and ordinary glass tubing, with a Pyrex Florence flask containing 35 to 75 c.c. (depending on the concentration of the urine) of 0.1 N sulphuric acid together with enough water to make a total volume of 150 c.c. and a drop or two of alizarin red solution. These delivery tubes are made from glass tubing, small enough to pass into the ready-made holes in the rubber stopper. For the sake of flexibility the delivery tube must consist of two parts connected with a short piece of rubber tubing. When these connections are made, apply the flame again at full force, but not directly under the center, until the acid and alkali in the flask have had time to mix. The contents in the flask begin to boil almost at once and 4 to 5 minutes boiling transfers the whole of the ammonia to the receiver. The contents in the receiver become heated but the degree is not of sufficient moment to cause any trouble.

After the distillation is complete, titrate the remaining acid in the receiving flask against 0.1 N sodium hydrate, a faint red color being accepted as the end-point, if the titration be conducted without previous cooling of the fluid. The color deepens on cooling, so that, when time permits, it is more satisfactory to cool in running water before titrating. The calculations are the same as in the preceding Kjeldahl method.

This method is not applicable to highly resistant materials, as for example milk, which cannot be completely destroyed within 6 minutes. Urines containing much sugar, also, belong in this class. If fuming sulphuric acid be substituted for ordinary sulphuric acid in the preparation of the hydrolyzing reagent, or if 2 c.c. of fuming sulphuric acid are used in addition to 5 c.c. of the regular reagent, sugar urines are readily destroyed within the heating period of 4 to 5 minutes. If this latter modification be used, 25 to 30 c.c. of saturated sodium hydrate must be added to the digested mixture before distilling off the ammonia.

Folin and Denis' Direct Nesslerization Method.

This method¹ combines the digestion of the urine, as in the preceding method, with the direct Nesslerization of the digestion mixture against a

¹ Jour. Biol. Chem., 1916, XXVI, 473. See, also, Pearce, Jour. Lab. and Clin. Med., 1916, II, 130.

known solution of ammonium sulphate. The presence of large amounts of sulphates in the digestion mixture was the chief obstacle in the way of this method, owing to the precipitation of colored mercury ammonium compounds used for the color comparison. For this reason, the results obtained by earlier workers have not been entirely satisfactory.¹

Solution and Apparatus Required.

1. *Nessler Reagent.*

Seventy-five grams of potassium iodid are dissolved in 50 c.c. of warm distilled water and 100 grams of mercuric iodid are added to this solution. Stir for a few minutes, when solution is complete, although it is not usually perfectly clear. Dilute this solution with 400 or 500 c.c. of water, filter, and make the filtrate up to one liter. This is the stock solution, from which a Nessler's solution of any required degree of alkalinity may be made as desired. For the determination of nitrogen by this method, the authors use a solution of about 2 per cent. alkalinity. To prepare this diluted Nessler reagent, proceed as follows: To 300 c.c. of the above double-iodid stock solution add 200 c.c. of 10 per cent. sodium hydrate, 500 c.c. of distilled water, and mix. (See Non-protein N. of blood.)

2. *Standard Ammonium Sulphate Solution.*

This must be prepared from an absolutely pure salt. The authors state that Kahlbaum's ammonium sulphate, labelled c. p. for analysis, is such and can be used after drying either over sulphuric acid for a day or two or by heating at 110 degrees C. for an hour before being weighed. 4.716 grams of this pure salt are weighed out and dissolved in 1 liter of 0.2 N solution of sulphuric acid (in order to keep out molds). This solution contains 1 mg. of nitrogen per c.c. This solution keeps well and is the stock from which weaker solutions are prepared, as desired, by appropriate dilution. For use in this method, the standard solution should be of such strength that it contains 1 mg. of nitrogen per 20 c.c., hence the stock solution must be diluted 20 times before being used.

3. *A 10 Per Cent. Solution of Sodium Hydrate.*

4. *Digestion Mixture.*

A filtered mixture of 100 c.c. of concentrated sulphuric acid, 300 c.c. of "syruy" (85 per cent.) phosphoric acid, and 15 c.c. of 10 per cent. copper sulphate solution.

5. *Pipets and Glass Ware.*

A calibrated long stem 1 c.c. Ostwald pipet, which will deliver exactly 1 c.c. when drained against the side of the test-tube and then blown clean, for measuring the urine; an ordinary 1 c.c. pipet for measuring the digestion mixture; hard glass test-tubes, preferably 190 mm. \times 15 mm.; volumetric flasks, capacity 100, 200 and 250 c.c.; microburners; a high grade colorimeter.

Technic.

Dilute the urine so that 1 c.c. contains from 0.7 to 1.5 mg. nitrogen. Urines having a specific gravity of 1018 or less should be diluted to one in

¹ Gulick, Jour. Biol. Chem., 1914, XVIII, 541; Bock and Benedict, Ibid., 1915, XX, 52; Taylor and Hulton, Ibid., XXII, 63.

five. Those of 1030 or over should be diluted to one in twenty. For urines ranging between 1018 and 1030 a dilution of one in ten is appropriate. With an Ostwald pipet measure into a hard glass test tube 1 c.c. of diluted urine. Add (with an ordinary pipet) 1 c.c. of the digestion mixture and a piece of granite to prevent bumping. Heat the tube over a microburner, with the bottom of the test-tube within 1 cm. of the top of the burner, until nearly all the water has been driven off as indicated by the absence of foaming and by the appearance of the denser sulphuric acid fumes. This should occur in 2 to 5 minutes. Cover the mouth of the test-tube with a watch glass and continue the heating with a flame so regulated that only a little of the acid fumes escape from the test-tube. In 0.5 to 3 minutes, counted from the time the test-tube was closed, the digestion should be clear and blue, green, or light straw yellow in color. Continue the heating for another 30 to 60 seconds, the total heating being in no case less than 2 minutes after the test-tube was closed.

Remove the flame and allow the test-tube to cool for 2 minutes. Add water, and rinse the contents of the tube into a 200 or 250 c.c. volumetric flask, using about 150 c.c. of water for this purpose. Determine the titratable acid content of 1 c.c. of the digestion mixture (as delivered by the pipet regularly used for measuring this solution), using 10 per cent. sodium hydrate with phenolphthalein as indicator. Add to the diluted digestion mixture in the volumetric flask 10 per cent. sodium hydrate solution in amount equal to $11/8$ or 1.375 times the titrating value obtained, plus 2 c.c. for alkalinity.

Into another volumetric flask, of the same capacity as that used above, introduce 1 c.c. of the digestion mixture and 20 c.c. of the standard ammonium sulphate solution (the stock solution diluted 20 times, hence 20 c.c. contains 1 mg.). Add about 125 c.c. of water and then the same amount of sodium hydrate as in the case of the unknown. Mix well. Now add, with a cylinder, to each volumetric flask 15 c.c. of the dilute Nessler's solution (see above) and mix quickly. Fill to the mark with water and mix thoroughly. Pour out a part of the unknown (as an additional precaution against incomplete mixing), and centrifuge, or filter through a small cotton plug a portion of the remainder for the colorimetric comparison. If the sediment obtained is mixed with a red deposit the Nesslerization has not been successful and the determination must be discarded. The liquid above the sediment (or the filtrate) must be crystal-clear, not the least bit "smoky."

Adjust the colorimeter, with the standard set at 20 mm. in both cups, until the two fields are as nearly alike as it is possible to get them. Then make two or three color comparisons with the standard against itself. When the result is accurate, replace the standard in one of the cups with the unknown and make one leisurely, careful reading. As the standard ammonium sulphate solution, as used, contains 1 mg. in 20 c.c., or 0.05 mg. in 1 c.c., the calculation of the unknown is as follows:

$$\frac{20}{\text{reading of unknown}} \times 0.05 = \text{mg. nitrogen in 1 c.c. of urine.}$$

(b) Urea (NH_2)₂CO.

A discussion of the various factors which have to do with urea excretion cannot be taken up at this time. The recent work in the laboratory of Hofmeister¹ as well as that of Chittenden and of Folin has shown us conclusively that it is no longer possible to consider the rate of urea formation as a direct measure of protein metabolism. It is without question true, as Leathes² has said, that the nitrogen, or a great part of it, may be removed from the protein, converted into urea, and expelled with the urine before the oxidation of the rest of the protein molecule has been started upon; and the fact that we can trace in the urine excreted in a given time all or the greater part of the nitrogen of the protein taken at a meal, tells us nothing whatever about the fate of that part of the protein which contains, it may be, as much as 80 or 90 per cent. of the total energy of the protein. Further, urea is not a measure of the true protein catabolism, because a great part of it is formed from nitrogen that has never been beyond the liver; and it is not the measure of the protein energy because it is largely derived from protein by reactions which leave the energy value of the molecules from which it originates but little altered. The importance of the denitrifying and desamidization reactions of the tissues must be much more considered in the future than they have been in the past.³

As usually stated in text-books, urea constitutes from 80 to 85 per cent. of the total nitrogen output. It has been customary to figure directly the amount of tissue protein which must have been decomposed in order to yield this amount of urea. These figures can hardly be taken as conclusive of such decomposition. Folin has shown that a definite exogenous as well as an endogenous protein metabolism occurs. With a patient upon a nitrogen-free diet, the urea constitutes only about 60 per cent. of the total nitrogen. This would represent the true endogenous urea formation. A diet which requires the patient to eliminate much higher percentages of urea is, therefore, causing increased systemic activity, but is not increasing the direct tissue decomposition, as this excess never becomes a part of the system. One of the most important laws of protein metabolism is that the amount of nitrogen in the body is not increased by, or not in proportion to, an increase in the nitrogen intake.

The amount of urea excreted on an average diet varies from 15 to 40 grams.⁴ Folin finds this excretion to be, on a diet of 119 grams of protein yielding 18.9 grams of nitrogen, 298. grams. On a nitrogen-free diet the amount of urea is 2.2 grams. This excretion will, of course, vary, depending upon the diet. Von Jaksch states that the excretion of urea bears a definite relation to the total nitrogen excretion, so that for clinical purposes direct urea determinations may well be dispensed with, as the correct urea-content of the urine may be found by multiplying the simple nitrogen of the urine by the

¹ Lang, Beiträge zur chem. Phys. und Path., 1904, V, 340.

² Problems in Animal Metabolism, Phila., 1906.

³ See Henriques and Anderson, Ztschr. f. physiol. Chem., 1913, LXXXVIII, 357; Ibid., 1914, XCII, 21; Davis, Bull. Johns Hopkins Hosp., 1915, XXVI, 154; Hoagland and Mansfield, Jour. Biol. Chem., 1917, XXXI, 487.

⁴ See McLean (Jour. Exper. Med., 1915, XXII, 212 and 366) for a discussion of the law governing the rate of urea excretion. See, also, Austin, Stillman and Van Slyke, Proc. Soc. Exp. Biol. & Med., 1919, XVII, 59; Nagayama, Am. Jour. Physiol., 1920, CI, 434 and 449; Marshall, Jour. Pharmacol. and Exp. Therap., 1920, XVI, 141; Fosse, Bull. soc. chim. biol., 1920, II, 4; Austin, Stillman and Van Slyke, Jour. Biol. Chem., 1921, XLVI, 91; Oliver, Jour. Exp. Med., 1921, XXXIII, 177.

factor 2. This statement should not be regarded seriously by the practitioner as the rule, as Folin shows, would require that 93.3 per cent. of the total nitrogen in the urine be in the form of urea.

Pathologic Variations.

The pathologic increase in the amount of urea is observed under the same conditions as those mentioned under an increase of total nitrogen. Thus in febrile conditions, in diabetes mellitus and insipidus,¹ after the resorption of an exudate, in malignant conditions, and in exophthalmic goiter, the urea may be markedly increased.

In conditions associated with destruction of hepatic parenchyma, or with a diminished rate of blood-flow through the liver, the urea excretion may be very considerably diminished.² Thus we find in acute yellow atrophy, carcinoma, cirrhosis, and phosphorus poisoning that the normal urea of the urine is replaced by other nitrogenous constituents. The normal function of the liver in converting ammonium compounds and amino acids into urea is so markedly interfered with that the urea may completely disappear from the urine in such cases. It should be mentioned that the liver is not wholly responsible for the conversion of the ammonia and amino acids into urea, as the kidney unquestionably plays some rôle in this process.³

In acute nephritis there may or may not be a diminution in the excretion of urea, depending upon the extent of the renal insufficiency. In the chronic types of nephritis we find that the urea excretion fluctuates to a great extent, periods of increase varying with those of decrease. In the early stages, even though large amounts of albumin and casts be present, the urea may be normal, while in the later stages it is often greatly diminished.

A diminished excretion of urea is observed in melancholia and in the advanced stages of general paresis, while in epilepsy and hysteria an increase or a decrease may be observed. In some cases of diabetes mellitus Hirschfeld has shown that the urea output may be diminished as the result of delayed absorption from the intestine. In these cases of diabetes the ammonia of the urine may be markedly increased owing to its combination with acid bodies and consequent withdrawal from hepatic activity.

While urea is a very important substance both clinically and chemically, it is very rarely tested by qualitative methods in medical work. The writer feels, therefore, that a description of the properties of this substance would best be learned by consulting works on physiologic chemistry.

Determination of Urea.

The methods for the determination of urea are numerous. Many of them are inaccurate although giving results clinically of importance. In the selection of a method for the determination of urea one must be governed entirely by the importance of the urea determination in any specific case. It

¹ See Bassler, *Jour. Am. Med. Assn.*, 1914, LXII, 282.

² See Fiske and Karsner, *Jour. Biol. Chem.*, 1913, XVI, 399.

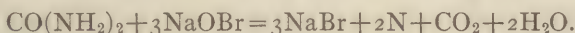
³ See Folin, *Jour. Biol. Chem.*, 1912, XI, 87; Van Slyke, *Ibid.*, 1913, XVI, 213; Marshall and Davis, *Ibid.*, 1914, XVIII, 53; Fiske and Sumner, *Ibid.*, 285; Jansen, *Ibid.*, 1915, XXI, 557. Palacios, *Am. Jour. Med. Sc.*, 1915, CXLIX, 267; Jansen, *Nederl. Tijdschr. v. Geneesk.*, 1916, II, 2271; Hammett, *Jour. Biol. Chem.*, 1919, XXXVII, 105.

should be said in advance that a urea estimation is absolutely useless unless taken in conjunction with the total nitrogen. The general practitioner usually insists on knowing both the percentage and total amount of urea excreted with utter disregard both for the nitrogen of the intake and of the output. If the urea is of any value at all it should be determined with these points in view. The prevailing idea is that a percentage output of two is approximately normal and he therefore bases his conclusion upon an increase or decrease with this as a standard. Not infrequently single voidings of urine are examined for the urea output. Such examinations are worse than useless and may even be harmful.

The methods for the determination of urea are distinctly separable into those useful for purely clinical purposes and those for the more exact metabolic work. The general practitioner, believing as he does in the importance of urea as an indicator of systemic activity and excretion, must have a rapid and easy method for estimation of urea. It is to be said, however, that the more exact methods would better be applied if reliable conclusions are to be drawn.

Knop-Hüfner Method.

The principle of this method is the decomposition of urea by means of sodium hypobromite and the measurement of the nitrogen evolved. Sodium hypobromite acts upon urea according to the following equation:



The carbon-dioxid evolved in this reaction is absorbed by the excess of alkali used, so that all that is necessary is to measure the amount of nitrogen evolved. This can be done by direct measurement or by collection in tubes which are so calibrated that each c.c. of nitrogen represents a certain percentage of urea.

Various forms of apparatus, which are termed ureometers, have been advised for the estimation of urea by this method. The form introduced by Knop and Hüfner is probably the most accurate, but is too complicated for general clinical purposes. As this method has absolutely no claim to accuracy it is useless in scientific investigations of the nitrogen partition of the urine. For clinical purposes, however, it serves as a rough approximation of the urea output and is, for this reason, largely used by the practitioner.¹ This method, applied as in the following discussion, is given merely because of the fact that it is almost the only method of estimating urea which can be carried out by the general practitioner.

Doremus Ureometer.

This instrument is seen in the accompanying cut. The graduations of the tube are such that the number of mg. of urea in the 1 c.c. of urine used in the test are directly read off instead of the number of c.c. of nitrogen formed in the reaction. As 0.01 gram of urea in one c.c. represents 1 gram per 100 c.c., this tube will furnish directly the percentage values of urea.

¹ See Krogh, *Ztschr. f. physiol. Chem.*, 1913, LXXXIV, 379; Robinson and Mueller, *Jour. Am. Med. Assn.*, 1914, LXII, 514; Golse (*Jour. Pharm. Chim.*, 1919, XIX, 20) has introduced a volumetric method based on this test. See, also, Philibert, *Ibid.*, 335, 386 and 434; Boyer, *Ibid.*, 346; Portes, Thesis, Montpellier, 1919; Philibert, Thesis, Paris, 1920; Seyot, *Ann. chim. anal. chim. appl.*, 1920, II, 11; Hurtley, *Biochem. Jour.*, 1921, XV, 11; Stehle, *Jour. Biol. Chem.*, 1921, XLVII, 11; Menaul, *Ibid.*, 1922, LI, 87; Stehle, *Ibid.*, 89.

The tube is filled with a solution of sodium hypobromite made by adding 1 c.c. of bromin to 40 c.c. of 20 per cent. cold sodium hydrate solution. This hypobromite solution decomposes after standing for a few days so that it is never wise to attempt to keep such a solution for any length of time. In the writer's laboratory a stock solution of 20 per cent. sodium hydrate is prepared and the bromin added to it only as occasion requires for preparing fresh solutions. In this way one will always have the material at hand and need have no fear of this stock solution decomposing. After filling the tube with this

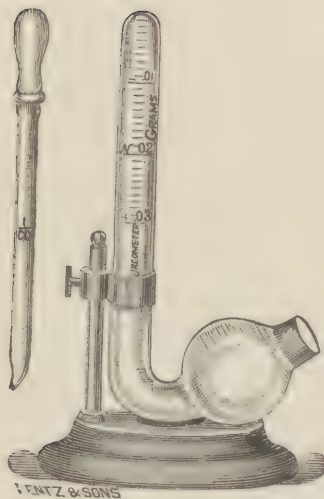


FIG. 73.—Doremus ureometer.

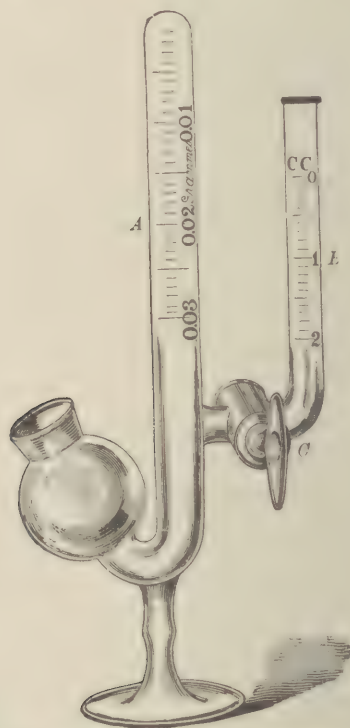


FIG. 74.—Doremus-Hinds ureometer.
(Hawk.)

hypobromite solution, 1 c.c. of urine is added by means of the curved pipet accompanying the instrument. In injecting the urine into the solution, the curved end of the pipet should be passed well under the curve of the bulb, the tube tilted slightly forward, and the urine forced into the hypobromite solution with a slow steady pressure. An evolution of gas will be observed at once and will cease in a short time. The carbon dioxide given off by the reaction of the urea upon the sodium hypobromite is absorbed by the excess of alkali and the nitrogen collects in the upper portion of the tube. As soon as the evolution of gas has ceased (5 to 10 minutes), the amount of urea is read off directly from the calibrations of the tube as previously described.

In this determination the urine should, theoretically, be free from both albumin and sugar. Practically, however, these substances are never re-

moved as the method yields comparative rather than accurate results. Indeed, a very delicate reaction for albumin is given by this test. One may be practically certain of the presence of albumin, if the urine, on being injected into the solution, forms rather characteristic heavy bubbles and if a dense froth collects in the upper portion of the tube. This froth settles slowly so that the percentage reading requires some time. The forms of this apparatus which substitute for the glass foot a wooden base are much to be preferred as they are not so easily broken. The modification of this instrument, as introduced by Hinds, is seen in the accompanying cut. In this form the urine is allowed to run in from the smaller graduated tube by opening the stop-cock. This modification is an advantage, but does not yield any more accurate results than does the preceding.

Folin's¹ Method.

The principle of this method is as follows: At a temperature of 160°C . crystallized magnesium chlorid ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) boils in its water of crystallization. If urea be present it is decomposed by this boiling solution into ammonia and carbon dioxide. If the conversion be carried out in acid solution, the ammonia formed will combine with the acid and may then be liberated by alkalinizing the mixture. The ammonia is distilled into a standard acid solution and may then be determined as given under the total nitrogen. In this process the preformed ammonia as also the trace present in the magnesium chlorid will also be determined so that a separate estimation of these factors must be made and subtracted from the total amount.

Technic.

Five c.c. of urine are measured into an Erlenmeyer flask of about 200 c.c. capacity, 5 c.c. of concentrated hydrochloric acid, 20 grams of crystallized magnesium chlorid, a piece of paraffin about the size of a hazelnut and two or three drops of a 1 per cent. aqueous solution of alizarin-red are added. An especially constructed safety-tube (see cut) is then inserted and the mixture boiled until the drops flowing back from the safety-tube produce a very perceptible bump or hissing sound on coming in contact with the solution (10 to 15 minutes). The temperature² is then somewhat reduced and the heating continued for one hour. It is important

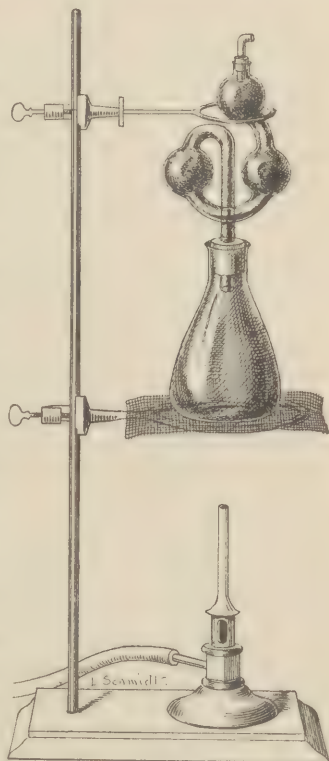


FIG. 75.—Folin's urea apparatus.
(Hawk.)

¹ Ztschr. f. physiol. Chem., 1901, XXXII, 504; Ibid., 1903, XXXVII, 548.

² Folin advises (Handb. d. Biochem. Arbeitsmeth., 1911, V, 286) the use of small glass bulbs containing solid HgCl_2 as an indicator of the proper temperature of this reaction. This substance melts at 153°C ., which is the optimum temperature to insure complete decomposition of the urea.

in this process that the reaction must not remain alkaline, and, therefore, as soon as the material in the flask turns red a very few drops of the acid distillate in the safety-tube are shaken back into the flask.¹ At the end of the hour the contents of the flask are washed into a liter Kjeldahl flask with about 700 c.c. of water. Twenty c.c. of 10 per cent. sodium hydrate are then added and the ammonia distilled, as described under total nitrogen, into a standard tenth-normal solution of sulphuric acid. This distillation should be continued until the contents of the liter flask are nearly dry or till the distillate shows no trace of ammonia with litmus paper. This will require about one hour. The distillate is then boiled to drive off the carbonic acid, is then cooled, and titrated with tenth-normal sodium hydrate to determine the amount of acid which combined with the ammonia formed. Alizarin red or methyl red are used as indicators. One c.c. of tenth normal sulphuric acid is equivalent to 0.001704 gram of ammonia (NH_3), or 0.001401 gram of nitrogen. If the nitrogen value be multiplied by 2.143 the result will be the amount of urea in the 5 c.c. of urine taken. From the total c.c. of $\text{n}/10$ sulphuric acid neutralized must be subtracted the $\text{n}/10$ sulphuric acid values for the preformed ammonia as well as for the ammonia which may be present as an impurity in the 20 grams of magnesium chlorid used.

It has been found by Schoorl that when carbohydrates and urea are heated together they form very stable condensation products (ureids). For this reason this method of Folin does not give accurate results with saccharin urine. A combination of this method with that of Mörner, which will be described later, will give absolutely accurate results. In the determination of the ammonia values of the preformed ammonia and of the magnesium chlorid, the later methods of Folin must be used.

Mörner-Sjöqvist Method.

This method² is an extremely accurate one, but no more so than that of Folin, except in saccharin urines. If albumin be present it must, however, be removed by heat and acetic acid and the original volume of the urine restored. If the urine contains large amounts of hippuric acid this method may not give accurate results, as Salaskin and Zaleski have shown.

Technic.

Five c.c. of urine are placed in a flask with 5 c.c. of baryta mixture consisting of a saturated barium chlorid solution containing 5 per cent. of barium hydrate. One hundred c.c. of a mixture of two parts of 97 per cent. alcohol and one part of anhydrous ether are then added and the mixture allowed to stand in a closed flask overnight. It is then filtered and the residue washed with fresh alcohol and ether mixture and the combined filtrates evaporated at a low temperature ($60^\circ\text{C}.$). Urea will be practically the only nitrogenous body left in solution, with the exception of traces of ammonia. When the evaporated filtrate has been reduced to about 25 c.c. in volume, a few c.c. of water and a small amount of calcined magnesium oxid are then added, the mixture stirred, and heated to drive off the ammonia; or the residue may be treated by Folin's method as previously described. This heating is con-

¹ See Folin, *Jour. Biol. Chem.*, 1912, XI, 507.

² *Skand. Arch. f. Physiol.*, 1891, II, 438; *Ibid.*, 1903, XIV, 247.

tinued until the vapor shows no alkalinity when tested with moistened litmus paper, a result usually obtained when about 10 to 15 c.c. of the mixture remain. The fluid and the residue are then washed into a Kjeldahl flask and treated with concentrated sulphuric acid, copper sulphate, and potassium sulphate as in the determination of total nitrogen. One part of nitrogen is equivalent to 2.143 grams of urea.

If much hippuric acid be present, Braunstein¹ advises oxidation of the urea by heating the evaporated residue with 10 grams of solid phosphoric acid (in an air-bath) to 140 to 145°C. for five hours. Alkalinize and distill as previously described. Benedict's² newer method also yields accurate results.

Urease Method.

Marshall has introduced a method for the determination of urea in various body fluids, which is based on an entirely different principle from the others advocated and which is accurate and extremely simple.³ He takes advantage of a fact, first discovered by Takeuchi, that an aqueous extract of soy bean (*glycine hispida*) contains a ferment which converts urea into ammonium carbonate and which neither acts upon anything else nor is interfered with by anything ordinarily found in the various body fluids. In his method Marshall employs this aqueous extract, but this has the disadvantage of requiring many hours to react quantitatively and, further, of losing its activity on standing for a few days, the loss being more rapid the higher the temperature at which it is kept. For these reasons the writer prefers to adopt the modification of *Van Slyke and Cullen*. These workers have produced a permanent preparation of this ferment by the following method: Extract soy bean meal with 5 parts of water and pour this extract into 10 volumes of acetone. Filter off the precipitate and dry it.⁴

This urease dissolves readily in water, forming an opalescent solution. "Under given conditions, a given amount of it will decompose a definite amount of urea per minute and no more, regardless of how much excess urea may be present. The preparation is most active at a temperature approximately 55°C. (131°F.) and in a perfectly neutral solution. Between 10 and 50°C. (50 and 122°F.), increasing the temperature over any 10° interval doubles the rate at which the preparation acts. Heavy metals, or acid in a relatively slight concentration, destroy the activity. The urease is not quite so sensitive toward an alkaline reaction, but its activity is decreased by even

¹ Ztschr. f. physiol. Chem., 1901, XXI, 381. See, Todd, Biochem. Jour., 1920, XIV, 252.

² Jour. Biol. Chem., 1910, VIII, 405. See, also, Milroy, Biochem. Jour., 1913, VII, 399.

³ Takeuchi, Jour. College Agriculture, Tokio, 1909, I, 1; Chem. Ztg., 1911, XXXV, 408; Marshall, Jour. Biol. Chem., 1913, XIV, 283; Ibid., 1913, XV, 495; Hahn and Saphra, Deutsch. med. Wchnschr., 1914, XL, 430; Van Slyke and Cullen, Jour. Am. Med. Assn., 1914, LXII, 1558; Jour. Biol. Chem., 1914, XIX, 141 and 211; Van Slyke, Zacharias and Cullen, Deutsch. med. Wchnschr., 1914, XL, 1219; Plimmer and Skelton, Biochem. Jour., 1914, VIII, 70; Hahn, Deutsch. med. Wchnschr., 1915, XLI, 134; Eigenberger Ztschr. f. physiol. Chem., 1915, XCIII, 370; Jacoby and Sugga, Biochem. Ztschr., 1915, LXIX, 116; Barendrecht, Proc. Acad. Sc. Amsterdam, 1910, XXI, 1126 and 1307; Ibid., XXII, 29; Le Goff, Report, pharm., 1919, XXX, 329; Wester, Pharm. Zentralh., 1920, LXI, 293; Carnot, Gerard and Moissonnier, Ann. de l'Inst. Pasteur, 1921, XXXV, 1; Lövgren, Biochem. Ztschr., 1921, CXIX, 215.

⁴ If one does not wish to prepare this urease for himself, he may obtain it from the Arlington Chemical Company of Yonkers, N. Y.; Mateer and Marshall, Jour. Biol. Chem., 1916, XXV, 297, have shown that the jack bean (*Canavalia ensiformis*) is about 15 times richer in urease than is the soy bean.

weak bases.¹ Ammonium carbonate depresses the activity to about one-eighth the optimum, so that the action of the enzyme is self-retarding.² The retarding effect of the ammonium carbonate formed can be neutralized and conditions for optimum activity obtained by the addition of potassium dihydrogen phosphate and dipotassium hydrogen phosphate in equimolecular proportions. This phosphate mixture also acts as a stabilizer to solutions of enzyme.”

Preparation of Enzyme Solution.

Two grams of the enzyme preparation, 0.6 gram of dipotassium hydrogen phosphate and 0.4 gram of potassium dihydrogen phosphate are stirred up with a rod in 10 c.c. of water. The preparation dissolves quickly, forming an opalescent solution. If this solution be covered with toluol it will hold its activity for a week or so, but it is better technic to employ relatively fresh solutions.

Technic.

This method employs a portion of the technic originally introduced for determination of ammonia by Folin (see page 253). Dilute 5 c.c. of urine with water to 50 c.c. Mix this thoroughly and transfer, by means of a pipet, 5 c.c. of this diluted urine to a clean, fairly heavy test-tube of about 50 c.c. capacity. Add 1 c.c. of the enzyme solution and 1 drop of caprylic alcohol or a few drops of benzol to prevent foaming during the later aeration. Close this tube (A) with a stopper and allow it to stand 15 minutes in order to permit the enzyme to act upon the urea present.

Into a second test-tube (B) measure with a buret 25 c.c. of fiftieth normal (N/50) hydrochloric or sulphuric acid and add 1 drop of a 1 per cent. solution of alizarin as an indicator and 1 drop of caprylic alcohol or benzol.

By means of glass and rubber tubing connect these two tubes in such a way that air may be forced through tube A into tube B (see page 253). After tube A has stood for 15 minutes, pass the air current for about $\frac{1}{2}$ minute in order to carry over into the acid any ammonia which may have passed into the upper portion of tube A. Now shut off the air, open tube A and pour into it 4 or 5 grams of dry potassium carbonate.³ Close tube A at once and pass the air current till all the ammonia is driven into tube B. This time depends on the rate at which the air is passed. With the ordinary apparatus this varies from 15 to 30 minutes.

When this process is finished, titrate the excess of acid in tube B with fiftieth normal (N/50) sodium hydrate, titrating to the appearance of the first permanent red point. Subtract the number of c.c. of standard alkali used from 25 (the number of c.c. of standard acid originally measured into tube B) and multiply this result by 0.056 to obtain the grams of urea nitrogen plus ammonia nitrogen in 100 c.c. of urine.

The ammonia nitrogen, which is not referable to the decomposition of urea, is obtained by the same method as the above, using 5 c.c. of undiluted urine

¹ Rona and Gy rj  (Biochem. Ztschr., 1920, CXI, 115) show that the optimum P_H for soy bean urease is 7.3-7.5.

² See Falk, Jour. Biol. Chem., 1917, XXVIII, 389; Van Slyke and Cullen, *Ibid.*, 391.

³ Fiske, Jour. Biol. Chem., 1915, XXIII, 455, advises the use of 5 c.c. of an alkaline solution prepared as follows: Dissolve 500 grams K_2CO_3 in 500 c.c. of water with slight heating; add 10 c.c. of a 30 per cent. solution of potassium oxalate, filter and allow to cool. This is concurred in by Van Slyke and Cullen, Jour. Biol. Chem., 1916, XXIV, 117.

and adding no urease solution. In this latter case multiply the number of c.c. of excess acid by 0.0056 instead of 0.056 to determine the percentage of ammonia nitrogen originally present in the urine. Subtract this latter figure from the figure for the combined nitrogen of urea and ammonia and obtain the true figure for the ammonia nitrogen derived from the action of the urease. If this nitrogen figure be multiplied by 2.143, one obtains the percentage of urea present as such.¹

Folin and Youngburg's Direct Nesslerization Method.

This method² is a modification of the method of Folin and Denis³ made necessary by the fact that Merck's blood charcoal, used in the latter method, is difficultly obtainable at the present time. It is a combination of the urease method just discussed with that of direct Nesslerization, as treated under Nitrogen, with some modifications. Instead of the charcoal, these authors use "Permutit," an aluminate silicate, zeolite, discovered by Gans.⁴

Technic.

Wash about 3 grams of permutit in a flask with 2 per cent. acetic acid, then twice with water; add 5 grams of fine jack bean meal⁵ and 100 c.c. of 30 per cent. alcohol. Shake gently but continuously for 10 to 15 minutes and filter. The filtrate contains practically the whole of the urease and extremely little of other materials. Add 1 c.c. of this urease solution to 1 c.c. of diluted urine⁶ (dilution usually 1:10) in a stoppered test-tube and digest in a beaker of warm water (40–55 degrees C.) for 5 minutes or at room temperature for 15 minutes. As a buffer mixture, add a drop of sodium pyrophosphate solution (140 grams of sodium pyrophosphate and 20 grams of glacial phosphoric acid per liter) at the beginning of the digestion. At the end of the digestion period, transfer the contents of the test-tube to a 200 c.c. volumetric flask, diluting to a volume of about 150 c.c.

To another 200 c.c. volumetric flask add 1 mg. of N in the form of ammonium sulphate (see Nitrogen Determination for the method of preparing this solution); to this standard add 1 c.c. of the urease solution and dilute to about 150 c.c.

To the solutions in each volumetric flask add 20 c.c. of the dilute Nessler solution discussed under Nitrogen or, on account of certain impurities in the mercuric iodid obtainable on the market, prepare the Nessler solution as follows:⁷ Transfer 150 grams of potassium iodid and 110 grams of iodine to a 500 c.c. Florence flask; add 100 c.c. of water and an excess (140 to 150 grams)

¹ Instead of using the method of aeration, one may titrate directly the ammonium carbonate formed by the action of the urease on the urea, using somewhat larger amounts of urine (5 c.c.) and titrating with N/10 hydrochloric acid, with methyl-orange as an indicator. This was the original method of Marshall, but it does not yield as accurate results as the technic described.

² Jour. Biol. Chem., 1919, XXXVIII, 111; see, also, Sumner and Bodansky, for a further Nesslerization method, *Ibid.*, 57.

³ Jour. Biol. Chem., 1916, XXVI, 501.

⁴ Jahrd. k. preuss. Geol. Landesanstalt, 1905, XXVI, 179; 1906, XXVII, 63. This substance is obtainable from the Permutit Company, 30 E. 42nd St., New York.

⁵ This meal, as also urease from the soy bean, may be obtained from the Arlington Chemical Company, Yonkers, N. Y.

⁶ Youngburg (Jour. Biol. Chem., 1921, XLV, 391) advises the removal of the urinary ammonia by treatment with permutit prior to the determination of urea.

⁷ Folin and Wu, Jour. Biol. Chem., 1919, XXXVIII, 89.

of metallic mercury. Shake the flask continuously and vigorously for 7 to 15 minutes or until the dissolved iodine has nearly disappeared. The solution becomes quite hot. When the red iodine solution has begun to become visibly pale, though still red, cool in running water and continue the shaking until the reddish color of the iodine has been replaced by the greenish color of the double iodide. This whole operation usually does not take more than 15 minutes. Now separate the solution from the surplus mercury by decantation and washing with liberal quantities of distilled water. Dilute the solution and washings to a volume of 2 liters. This is the stock Nessler solution, from which the dilute form is prepared as follows: Introduce into a large bottle 3500 c.c. of 10 per cent. sodium hydrate solution, add 750 c.c. of the double iodide stock solution and 750 c.c. of distilled water.

After the addition of the dilute Nessler solution, dilute the mixture in the volumetric flasks to volume and make the color comparisons in the colorimeter, as discussed under Nitrogen. The ammonia nitrogen, due to the preformed ammonia of the urine is, of course, included in the figure obtained and must be determined, as outlined later, and deducted from this figure in order to arrive at the exact nitrogen value referable to urea.

The authors call attention to the fact that there are many kinds of biological nitrogenous materials, particularly amino-acids, peptones, and albumins, which prevent the development of color reactions given by ammonia and Nessler's reagent. The color in such cases tends to be visibly more greenish and less distinctly red than the standard. Correct results are, however, given by this method even with albuminous urines. Further attention is called to the fact that the glass ware used in this test must be washed thoroughly with nitric acid or, as others advocate, with potassium iodide and alkali, in order to remove traces of mercury compounds which may adhere to the glass.¹

(c) **Ammonia (NH₃).**

This substance, although chemically belonging in the class of inorganic compounds, is so closely related to the nitrogen metabolism that it is more properly discussed under the heading of Nitrogenous Bodies.

Ammonia is one of the most important products of protein metabolism. It is constantly present in small amounts in normal urine averaging about 0.85 gram of NH₃ in 24 hours, representing from 4 to 5 per cent. of the total nitrogen. It is present in combination with various acids and may represent largely a portion of the nitrogen which has not been transformed into urea, but has been used to combine with acid substances formed in the protein metabolism of the body. Any increase in the production of acid in the system or any increased intake of noncarbonate-forming acids will lead to an increased excretion of ammonium salts. This is an important factor in the metabolism of conditions associated with an acidosis.

The total output of ammonia will vary under normal conditions with the diet or, in other words, with the intake of total nitrogen.² While the

¹ For other methods employing urease see Kennaway, *Brit. Jour. Exp. Path.*, 1920, I, 135; Roman, *Jour. Urol.*, 1920, IV, 531; Malerba, *Rif. Med.*, 1921, XXXVII, 362.

² See Tanji, *Deutsch. Arch. f. klin. Med.*, 1914, CXVI, 92; Wills and Hawk, *Jour. Am. Chem. Soc.*, 1914, XXXVI, 158, have shown an increased output of ammonia following and proportional to increased ingestion of water.

increase of the total nitrogen of the urine or increased nitrogen intake is largely in the form of urea, yet a small increase in the absolute amount of ammonia must occur. Likewise we observe a diminished intake of nitrogen reducing the absolute value of ammonia, but largely increasing its relative value. Thus Folin finds with a total excretion of 16 grams of nitrogen, an ammonia output of 0.85 gram (4.3 per cent.); while on a nitrogen-free diet a total nitrogen output of 3.6 grams was observed with an ammonia elimination of 0.51 gram (11.3 per cent.). We therefore conclude with Folin as follows: "With pronounced diminution in the protein metabolism (as shown by the total nitrogen in the urine), there is usually, but not always, and therefore not necessarily, a decrease in the absolute quantity of ammonia eliminated. A pronounced reduction of the total nitrogen is, however, always accompanied by a relative increase in the ammonia nitrogen, provided that the food is not such as to yield an alkaline ash."

Although the ammonium salts of many organic acids are converted into urea in the system we find the ammonium salts of the sulphuric and phosphoric acids formed in the decomposition of protein material are excreted as such. Moreover, we observe that an increased consumption of fat, either taken in as food or derived from the tissue, is associated with a combination of ammonia with the fatty acids. This provision of metabolism, by which the system is protected against the deleterious effects of increased acidity by neutralization of acid compounds with ammonia, is of the greatest importance, as the fixed alkalies of the tissues are thereby maintained in their usual concentration unless the pathologic processes be extreme.

Pathologic Variations.

An increased output of ammonia is observed in cases of diminished oxidative powers of the system, in febrile diseases, in hepatic disturbances such as carcinoma and acute yellow atrophy, in uremia, in acid intoxication, in dyspnea from any cause, in the toxic vomiting of pregnancy, in delayed chloroform poisoning, and especially in diabetes mellitus. In this latter condition the degree of acidosis may be conveniently followed by watching the ammonia output.

A reduction in the amount of ammonia is observed in many cases of nephritis and in some cases of carcinoma of the stomach, although there is at the same time a diminished excretion of hydrochloric acid in the gastric contents. Edsall reports a reduction in cases of periodic insanity preceding the attack, while a rise is observed as the attack proceeds. Administration of large doses of the fixed alkalies will usually diminish the ammonia output.¹

Quantitative Determination of Ammonia.

Like the methods given under Urea, many have been advanced for the determination of the urinary ammonia. One of these, though inaccurate, has been so long used and even to-day is so relied upon in many quarters that

¹ See Janney, *Ztschr. f. physiol. Chem.*, 1912, LXXVI, 99; Palmer and Henderson, *Arch. Int. Med.*, 1915, XVI, 109; Denis and Minot, *Jour. Biol. Chem.*, 1918, XXXV, 101. Marriott and Howland, *Arch. Int. Med.*, 1918, XXII, 477; Campbell, *Biochem. Jour.*, 1920, XIV, 603; Cullis and Hewer, *Ibid.*, 757; Kretton, *Jour. Biol. Chem.*, 1921, XLIX, 411;

the writer includes it with the understanding that he advocates only the accurate methods. If the ammonia is worth determining, definite results should be sought and hence the most accurate methods are the ones to be used. They are no more complicated, not as time-consuming, and give more reliable results.

Method of Schlösing.

This method is the one most commonly used, but is open to the objection that it does not yield accurate results and is time-consuming, but has the advantage of simplicity. It is argued by many that the element of time is of no importance, as clinically one would not wait for an ammonia determination before instituting vigorous treatment. On the other hand, in metabolic work it is of a great advantage to get the work out of the way as quickly as is possible and consistent with accurate results.

Technic.

Twenty-five c.c. of urine are placed in the vessel B (preferably a Petri dish) (see cut). Above this is placed a glass triangle upon which rests a dish

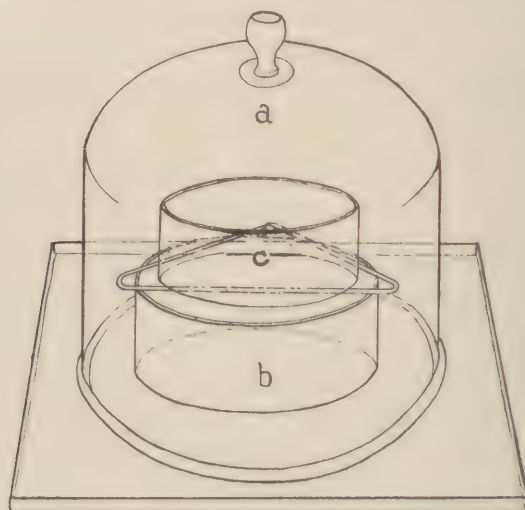


FIG. 76.—Schlösing's ammonia apparatus.

(C) containing 20 c.c. of tenth-normal sulphuric acid. Twenty c.c. of milk of lime are then poured into the dish containing the urine and the whole covered with a bell-jar, the borders of which have been well greased to make an air-tight union when the jar is placed upon the glass plate. This apparatus is then allowed to stand at room temperature from four to five days, during which time the ammonia, liberated by the action of the milk of lime upon the ammonium salts of the urine, will be taken up by the sulphuric acid in the vessel C. At the end of this time the bell-jar is removed, the acid titrated with tenth-normal sodium hydrate, and the number of c.c. of remaining acid determined. One c.c. of tenth-normal sulphuric acid neutralized by the evolved ammonia represents 0.001704 gram of ammonia. This figure is

multiplied by 4 to obtain the percentage ammonia value. If any moisture is present on the inside of the bell-jar it should be washed into the sulphuric acid before titration.

This method, as previously stated, does not give accurate results owing to the fact that ammonia may be split off from urea and thus give figures which are somewhat high. It has been found that if the apparatus be kept at $38^{\circ}\text{C}.$, the time necessary for this reaction may be reduced to 48 hours. If we add to the urine instead of the milk of lime, 0.5 gram of sodium carbonate and about 10 grams of sodium chlorid, no ammonia will be split off from the urea and no decomposition of the urine will occur (Schaffer).

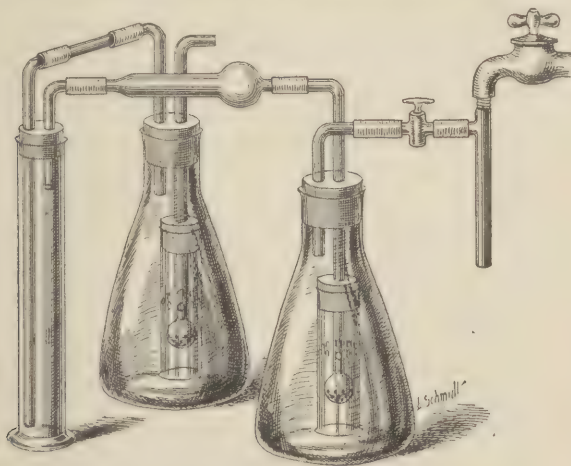


FIG. 77.—Folin's ammonia apparatus. (Hawk.)

Folin's Method.

The ammonia in this method¹ is set free by the addition of a weak alkali (sodium carbonate), is then removed from the urine at ordinary room temperature by means of a strong air-current, is collected in tenth-normal sulphuric acid and then titrated.

Technic

Twenty-five c.c. of urine are poured into an aerometer cylinder (30 to 40 cm. high), 15 grams of potassium oxalate,² 1 gram of dry Na_2CO_3 and some crude petroleum (to prevent foaming) are added. The upper end of the cylinder is then closed by means of a doubly perforated rubber stopper, through which pass two glass tubes, only one of which is long enough to reach below the surface of the liquid.³ The shorter tube (about 10 cm.) in length is connected

¹ Ztschr. f. physiol. Chem., 1902, XXVII, 161; Ibid., 1903, XXIX, 477. See Halin and Kootz, Biochem. Ztschr., 1920, CV, 220.

² Jour. Biol. Chem., 1910, VIII, 497; Denison, Jour. Lab. and Clin. Med., 1917, II, 204.

³ Folin and Macallum (Jour. Biol. Chem., 1912, XI, 523) have originated a micro-chemical method, which permits of much quicker estimation. 1 to 5 c.c. of urine, depending on the amount suspected, are placed in a large Jena test-tube by means of an Ostwald pipet. Add to the urine a few drops of a solution containing 10 per cent. potassium carbonate and 15 per cent. potassium oxalate and follow this with a few drops of kerosene or benzol to prevent foaming. Pass the strong air current for 10 minutes, collecting the ammonia in a 100 c.c. volumetric flask containing about 20 c.c. of water and 2 c.c. of N/10 hydrochloric acid. Nesslerize this solution against a standard ammonium sulphate solution as described in section on Blood or titrate it with N/50 alkali using methyl-red as an indicator.

with a calcium chlorid tube filled with cotton, which in turn is connected with a glass tube extending to the bottom of a wide-mouthed bottle (capacity about 500 c.c.) which contains 20 c.c. of tenth-normal sulphuric acid, 200 c.c. of water and a few drops of an indicator (alizarin-red). The complete absorption of the ammonia by the sulphuric acid is most easily insured by the use of a simple absorption tube which compels a very intimate contact of the air coming from the cylinder with the acid and water in the absorption bottle. This absorption bulb consists of a glass tube, measuring about 8 mm., in diameter, one extremity of which has been blown into a small bulb. By means of a heated platinum wire, 10 to 12 holes, each about 1 mm. in diameter, are made in this bulb.

The absorption bottle is then attached by means of a glass tube and rubber connection to a filter pump which will draw 700 liters of air per hour.¹ The air passing through the alkaline urine will draw the ammonia into the standard acid in from one and one-half to two hours. In order to exclude

any error due to the presence of ammonia in the aspirated air, a similar absorption apparatus is attached to the distal side of the evolution flask. The amount is then determined by titration of the standard acid with tenth-normal sodium hydrate, using methyl-red as an indicator. Steel, Gies and others advise the use of NaOH instead of Na_2CO_3 in this method on the ground that the latter will not liberate NH_3 from ammonium magnesium phosphate. This would not seem to militate against the method as the triple phosphate is rarely, if ever, present in the urine in appreciable amounts except when the urine is decomposed. Under such circumstances the ammonia factor would be useless from the metabolic standpoint.

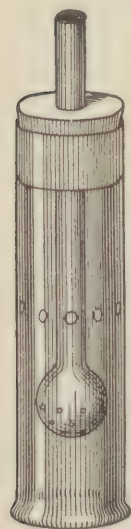


FIG. 78.—
Folin's absorp-
tion bulb.
(Hawk.)

Folin and Bell's Direct Nesslerization Method.

This method² is a modification of that of Folin and Denis³ in which permittit is used to absorb the creatinin, which interferes with the Nesslerization process, instead of the Merck's blood charcoal, which is almost nonobtainable. The removal of ammonia by this mineral is not an adsorptive phenomenon. The chemical affinity of the active group in the reagent for ammonia is remarkably strong, so that under suitable conditions the exchange becomes quantitative as far as the ammonia is concerned. This absorption is best from neutral solutions, but is also good from weakly acid solutions. The presence of much acid is not good because the reagent is dissolved by acids. In alkaline solutions ammonia is not absorbed. The serviceability of the reagent depends, fundamentally, on the fact that in the presence of sodium hydrate

¹ In laboratories in which compressed air is available, the ammonia may be forced from the generating cylinder into the cylinder containing the standard acid. In this case, a cylinder containing 100 c.c. of 10 per cent. sulphuric acid is interposed between the air blast and the generating cylinder in order to catch any ammonia which may be present in the air.

² Jour. Biol. Chem., 1917, XXIX, 329.

³ Ibid., 1916, XXVI, 497; see, also, Sumner, Ibid., 1918, XXXIV, 37.

the absorbed ammonia is again set free. This reagent does not appreciably deteriorate by being used. After washing away the Nesslerized ammonia and surplus alkali first with water, then with one portion of 2 per cent. acetic acid, then once more with water, the powder remaining is efficient and usable.

Technic.

Transfer about 2 grams of the permutit (either fresh or well washed, as above) to a 200 c.c. volumetric flask. Add about 5 c.c. of water (no more) and with an Ostwald pipet introduce 1 or 2 c.c. of urine, or with a 5 c.c. pipet introduce 5 c.c. of previously diluted urine (corresponding to 1 or 2 c.c. of original urine). Rinse down the added urine by means of a little water (1 to 5 c.c.) and shake gently but continuously for 5 minutes. Rinse the powder to the bottom of the flask by the addition of water (25 to 40 c.c.) and decant. Add water once more and decant. (In the case of urines rich in bile it is advisable to wash once or twice more.) Add a little water to the powder, introduce 5 c.c. of 10 per cent. sodium hydrate solution, mix, then add more water until the flask is about three-fourths full. Shake for a few seconds and then add 10 c.c. of Nessler's reagent (prepared as directed under total nitrogen and urea). Mix and let stand for 10 minutes or longer. Fill up to the mark with water, mix, and make the color comparison in the colorimeter with the standard ammonium sulphate solution. This standard solution is prepared as directed under total nitrogen and should be, in this case, 1 mg. or, less frequently, 0.5 mg. of ammonia nitrogen, Nesslerized as above with the addition of 5 c.c. of 10 per cent. sodium hydrate solution and 10 c.c. of the dilute Nessler's solution, diluted to 200 c.c.

Formalin Method.

This method, originated by Ronchèse¹ and Malfatti,² depends on the fact that a solution of an ammonium salt, treated with formaldehyd, decomposes with the formation of hexamethylen-tetramin, the acid combined with the ammonia being liberated. This can then be determined by titration.

Dilute 10 c.c. of urine with 50 c.c. of water, add 2 or 3 drops of a 1 per cent. alcoholic solution of phenolphthalein and neutralize with N/10 NaOH. Five c.c. of formalin, previously neutralized with N/10 NaOH, are added to the neutralized urine and the mixture is again titrated with N/10 NaOH to the appearance of a faint, permanent pink color, the amount of alkali used being noted. It is evident that 1 c.c. of this N/10 NaOH is equivalent to 1 c.c. of N/10 NH₃ or, in other words, represents 0.001704 gram of NH₃. Multiply the number of c.c. of N/10 NaOH used by this factor and obtain the NH₃ in 10 c.c. of urine.

(d) Uric Acid, C₅H₄N₄O₃.

In times past uric acid has been credited with much more clinical importance than is to-day ascribed to it. It is not, as many believe, a product of protein decomposition, as such, but can be derived only by the splitting of

¹ Jour. Pharm. et Chim., 1907, XXV, 611; Bull. Soc. Chim. de France, 1907, I. 000.

² Ztschr. f. anal. Chem., 1908, XLVII, 273.

been abandoned. However, we do know, from the work of Frerichs and Wöhler that the system does transform a certain amount of uric acid into urea.

Burian and Schur point out that the uric acid eliminated by man on a purin-free diet (endogenous uric acid metabolism) is for each individual a constant quantity and entirely independent of the total amount of nitrogen eliminated. This fact has so firmly fixed itself in the minds of the profession that the important results of Folin have been largely unnoticed. This latter worker finds that "when the total amount of protein metabolism is greatly reduced, the absolute quantity of uric acid is diminished, but not nearly in proportion to the diminution in the total nitrogen, and the per cent. of the uric acid nitrogen in terms of the total nitrogen is, therefore, much increased." It would seem, therefore, that this point is still very debatable. Quoting again from Folin "if the endogenous uric acid is to be considered as derived from the cell nucleins exclusively, it would, indeed, seem highly plausible that the quantity should tend to remain constant, even with very great variations in diet. Rigid proof that the endogenous uric acid elimination is for each individual a constant quantity would be strong evidence in favor of such a theory. Burian and Schur support the view that the endogenous uric acid is derived from the cell nucleins, but they contend that in man about one-half of the uric acid so derived is destroyed inside the organism and that only the other half is eliminated. With the introduction of this important modification of the nuclein theory, there is no longer any reason why the uric acid elimination should not be a decidedly variable factor which might well be susceptible to change under the influence of many different changes in the conditions, among others, changes in diet." It will be seen, therefore, that one must be on his guard in drawing conclusions from the amount of uric acid in the urine, as to the degree of nuclein decomposition within the system.¹

Physiologic Variations.

The output of uric acid varies, depending upon the diet, from 0.2 to 2 grams in 24 hours. On a diet of 119 grams of protein with a total urinary nitrogen output of 16 grams the uric acid eliminated was 0.37 gram (0.8 per cent. of total N); while on a nitrogen-free diet with a urinary nitrogen of 3.6 grams the output of uric acid was 0.09 gram (2.5 per cent. of total N). It is increased physiologically by increase in the nucleins of the diet, sweet-breads, liver, kidneys, and brain yielding very large amounts of uric acid. A meat diet will lead to a larger excretion of uric acid than will a vegetable diet, the maximum output being observed about five hours after a hearty protein meal. According to Horbaczewski, this increase is dependent upon the leucolysis which occurs at the time of the disappearance of the digestive leucocytosis.

¹ See Siven, Arch. f. d. ges. Physiol., 1912, XCLV, 283; Ibid., 1914, CLVII, 582; Faustka, Ibid., 1914, CLV, 523; Raiziss, Dubin and Ringer, Jour. Biol. Chem., 1914, XIX, 473; Daniels and McCrudden, Arch. Int. Med., 1915, XV, 1046; von Moraczewski and Herzfeld, Ztschr. f. klin. Med., 1915, LXXXII, 61; Umeda, Biochem. Jour., 1915, IX, 421; Mendel and Stehle, Jour. Biol. Chem., 1915, XXII, 215; Fine, Ibid., XXIII, 471; Wells, Ibid., 1916, XXVI, 319; Neuwirth, Ibid., 1917, XXIX, 477; Lewis and Doisy, Ibid., 1918, XXXVI, 1; Lewis, Dunn, and Doisy, Ibid., 9; Gudzent, Maase and Zondek, Ztschr. f. klin. Med., 1918, LXXXVI, 34; Höst, Jour. Biol. Chem., 1919, XXXVIII, 17.

The amount of exercise will also influence the output in the urine; while an intake of a large amount of water will increase the normal uric acid value. A certain relation between the amount of urea and of uric acid excreted seems to obtain. As a rule, it may be said that the nitrogen of the urea is to the nitrogen of the uric acid as 50 or 60 to 1. It is probable that variations in this relation have at present much less clinical value than in times past. It does not seem to the writer consistent to assume a special "uric acid diathesis" if this normal relation be disturbed in the sense that the uric acid is increased.

Pathologic Variations.

A pathologic increase of uric acid is observed whenever we have increased protein catabolism. Thus, in fever, the output of uric acid runs parallel to that of urea. In cases associated with marked leucocytosis a large increase in the uric acid output may be noted, which is referable to the constant leucolysis. This increase is especially marked in leukemia, an output of more than 12 grams in 24 hours having been observed by Magnus Levy. In cases of pneumonia, associated with a high leucocyte count, an increase in the uric acid output is seen, being especially marked after the crisis, but may even precede the crisis.

Gout¹ has so long been associated, in the minds of the profession, with increased uric acid formation that such a relationship is generally accepted. It is true that during the acute attack the blood may contain an increased amount of uric acid, but never in such a large amount as in cases of leukemia, for instance. The mere excess of uric acid in the blood can, therefore, not be the determining factor. As our knowledge of the true etiology of gout is so obscure the writer will not attempt a discussion of uric acid in such relations. According to Fitcher the uric acid is below the normal standard preceding the acute attack, rises to much increased values during the attack, and again falls below the normal limit after the subsidence of the acute symptoms. In acute articular rheumatism an increased elimination is noted during the febrile period, a decrease being observed as convalescence approaches. In diabetes mellitus an increase or a decrease may be observed, the uric acid excretion varying inversely as that of the sugar, giving rise to the term *diabetes alternans*. In cases showing much degeneration of hepatic tissue, as in cirrhosis and acute yellow atrophy, uric acid may be largely increased.

A diminished excretion of uric acid is usually observed in the ordinary forms of anemia while in pernicious anemia an increase may be noted. Chronic interstitial nephritis, chronic lead-poisoning, purpura hemorrhagica, and some cases of epilepsy, are associated with a diminished output of uric acid. Large doses of quinin or of opium may diminish the uric acid, while salicylic acid, chnic acid, colchicin, urotropin, piperazin, nucleinic acid, etc., may increase the amount.²

The chemical properties of uric acid may be found in works on physiologic chemistry. Qualitative tests are frequently desirable for the detection

¹ See Benedict, Jour. Lab. and Clin. Med., 1916, II, 1; Yavein, Russk. Vrach, 1916, XV, 1203; McClure and Pratt, Arch. Int. Med., 1917, XX, 481.

² See Denis, Jour. Pharmacol. and Exper. Therap., 1915, VII, 601; Haskins, Arch. Int. Med., 1915, XVI, 1055; Ibid., 1916, XVII, 405; Jour. Biol. Chem., 1916, XXVI, 205.

of the nature of certain deposits, as, for instance, renal infarcts. These will be discussed in a later section in association with the description of the various crystalline forms which uric acid may assume in a urinary deposit.

Quantitative Determination.

Folin and Shaffer's Method.

This modification¹ of Hopkins' method depends upon the precipitation of uric acid, as ammonium urate, by addition of ammonium sulphate. The urate is decomposed with sulphuric acid and the liberated uric acid is determined by titration with a standard solution of potassium permanganate.

Technic.

Two hundred c.c. of urine are treated with 50 c.c. of a reagent consisting of 50 grams of ammonium sulphate, 5 grams of uranium acetate, and 60 c.c. of 10 per cent. acetic acid, dissolved in 650 c.c. of water. The mixture is allowed to stand without stirring for about one-half hour. The precipitate of uranium phosphate has then settled and the clear supernatant liquid is removed by siphonage or by decantation. One hundred and twenty-five c.c. of this clear fluid, representing 100 c.c. of urine, are measured into a beaker, 5 c.c. of strong ammonia are added, and the mixture set aside until the following day. The precipitate of ammonium urate, which is produced by alkalinizing the solution saturated with ammonium sulphate, is then filtered off, and washed with 10 per cent. ammonium sulphate solution until the filtrate is practically free from chlorids. The filter is removed from the funnel, opened, and the precipitate rinsed back into the beaker, enough water to make about 100 c.c. being used. The precipitate is now dissolved by adding 15 c.c. of concentrated sulphuric acid. The solution is then titrated with a twentieth-normal solution of potassium permanganate, until the first pink coloration is observed extending through the entire liquid after the addition of two drops of the permanganate solution. Each c.c. of potassium permanganate used corresponds to 3.75 mg. of uric acid. Owing to the solubility of the ammonium urate, a correction of 3 mg. per 100 c.c. of urine must be made. The corrected result gives the percentage of uric acid.²

A method of making a twentieth-normal potassium permanganate solution may be found in any work in general chemistry. The student is to be cautioned that a twentieth-normal solution of potassium permanganate, as used in this connection, has reference to one of such a concentration that one liter would contain 0.05 gram of available oxygen for oxidizing purposes. This solution is obtained by dissolving 1.581 grams of pure KMnO_4 in one liter of water. As this weighing may not be sufficient, owing to slight impurities in the permanganate, it is wise to titrate this solution against a known twentieth-normal solution of oxalic acid. If the solutions correspond, 1 c.c. of each should be the equivalents. The titration of the oxalic solution is made by measuring out 10 c.c. of oxalic acid solution, diluting to approximately 100 c.c. with distilled water, and adding 15 c.c. of concentrated sulphuric acid. The

¹ *Ztschr. f. physiol. Chem.*, 1901, XXXII, 552. See Morris, *Jour. Biol. Chem.*, 1919, XXXVII, 231, for a new titration method involving the use of potassium permanganate.

² Skorczewski und Sohn (*Wien. klin. Wchnschr.*, 1911, XXIV, 1700) show that the urine of patients taking atophan may reduce the permanganate and, hence, introduce an error.

permanganate solution is then added drop by drop until a faint permanent red color is observed throughout the liquid, which does not disappear on stirring but persists for at least 30 seconds. If these solutions do not correspond the permanganate solution must be diluted according to the formula previously given under the determination of the chlorids in the urine.

Salkowski-Ludwig Method.

This method is not as convenient as that of Folin, but is inserted as it serves as a combined method of determining uric acid and the purin bases at the same time.

The principle is as follows: Uric acid is precipitated, in the form of a double urate of silver and magnesium, by an ammoniacal silver solution in the presence of magnesium salts. The silver is removed by hydrogen sulphid and the uric acid precipitated by hydrochloric acid, after which it may be estimated by direct weighing or by determination of the nitrogen.

Technic.

Two hundred and forty c.c. of urine are treated with 60 c.c. of magnesium mixture, which is made up as follows: One hundred grams of crystallized magnesium chlorid and 200 grams of ammonium chlorid are dissolved in about 500 c.c. of water, ammonia is added until the mixture smells strongly of this substance and the whole is then made up to one liter. The above mixture is well stirred and immediately filtered through a dry filter-paper into a beaker. Two hundred and fifty c.c. of filtrate (representing 200 c.c. of urine) are then measured off and treated with 10 to 15 c.c. of an ammoniacal silver nitrate solution (one liter of which contains 26 grams of AgNO_3 and enough ammonia to dissolve the precipitate formed). The precipitate should be of a flocculent gelatinous nature and of a yellowish color. If the precipitate be white, too much silver chlorid is present and more ammonia must be added. The clear solution above the precipitate should contain an excess of silver chlorid which may be shown by adding a little nitric acid to a few drops of the clear supernatant liquid. This mixture is then filtered, any particles adhering to the beaker being transferred to the filter by means of water and a rubber-tipped glass rod. The residue on the filter is washed with distilled water until the wash fluid shows no trace of silver or of chlorids. The funnel is now placed in the neck of a liter flask, the tip of the filter-paper is perforated with a glass rod, and the precipitate washed into the flask and thoroughly mixed with the water. This solution is made faintly acid by the addition of two or three drops of hydrochloric acid. Three to four c.c. of 10 per cent. copper sulphate solution are then added and the mixture boiled, after which hydrogen sulphid is passed through the hot mixture to precipitate the silver salts. This gas should be passed until the solution is saturated with it, after which the solution is boiled and filtered. The precipitate is washed with hot water and the filtrate, which must be clear and colorless, is evaporated to a small bulk (10 to 15 c.c.). Ten to fifteen drops of hydrochloric acid are then added, the mixture stirred and allowed to stand for a few hours, preferably over night. This addition of hydrochloric acid precipitates the uric acid and leaves the purin bases in solution. The crystals of uric acid are filtered

off on a small weighed filter, are washed with water slightly acidified with HCl to such an extent that the total wash-water and filtrate should not be more than 50 to 60 c.c. The precipitate is washed with alcohol, carbon disulphid, and ether, and is then dried and weighed. The difference between the original weight of the filter-paper and that of the paper and precipitate is the amount of uric acid in the 200 c.c. of urine. Owing to the slight solubility of uric acid in acidulated water, a correction of 0.00048 gram must be added for every 10 c.c. of the filtrate and wash-water.

Instead of weighing the uric acid, the filter-paper and contents may be placed in a Kjeldahl flask and a nitrogen determination made as previously described. The nitrogen value multiplied by 3 will give the weight of uric acid in 200 c.c. of urine.

The filtrate and wash-water from the uric acid precipitation contains the purin bases. This filtrate is alkalized with ammonia and again precipitated with the ammoniacal silver solution. This precipitate is collected on a small filter, washed with water, dried and carefully incinerated. The ash is dissolved in nitric acid and the silver chlorid estimated by titration with potassium sulphocyanate as described under the determination of chlorids. One c.c. of the potassium sulphocyanate solution is equivalent to 0.00734 gram of silver. One part of silver, in the form of the silver compounds of the purin bases, represents 0.277 gram of nitrogen, or 0.7381 gram of the purin bases. It is evident, therefore, that 1 c.c. of the potassium sulphocyanate solution will represent 0.002 gram of nitrogen and 0.00542 gram of purin bases according to the following proportion:

$$1 : 0.00734 :: 0.277 : x$$

$$x = 0.002$$

By multiplying the number of c.c. of potassium sulphocyanate solution used to precipitate the chlorids by 0.00542 we obtain the number of grams of purin bases in the 200 c.c. of urine originally used. It is wise in determining the amount of purin bases to start with a larger quantity of urine, as, for instance, 600 to 700 c.c., as the amount present in 200 c.c. would be very small.

This method is apt to give slightly high values for uric acid, as the purin bases may not be entirely soluble in the acid solution used to separate them from the uric acid. It requires much more equipment than is usually at the disposal of the practitioner so that it can hardly be recommended for routine use in general work.

Method of Rudisch and Kleeberg.

This method¹ is quite as accurate as is the preceding and has the advantage that it can be carried out in from 20 to 30 minutes. The principle is as follows: The total purin bodies are precipitated by an excess of silver nitrate, and the excess of silver determined volumetrically² by potassium iodid, using a mixture of nitrous and sulphuric acid with starch solution as an indicator. As the silver compounds of the purin bases are soluble in strong ammonia solution, it is possible to make a determination of the uric acid and then esti-

¹ Am Jour. Med. Sci., 1904, CXXVIII, 899.

² See Kretschmer, Biochem. Ztschr., 1913, L, 223.

mate the purin bases by subtraction of this value from the total purin compounds.

Technic.

One hundred and ten c.c. of urine are treated with 55 c.c. of fiftieth-normal AgNO_3 solution and diluted with strong ammonia to 220 c.c. (The fiftieth-normal solution of silver nitrate is made by dissolving 3.3932 grams of AgNO_3 , which has been heated for 10 minutes with a small amount of water to 120°C ., in 500 c.c. of water, adding 75 c.c. of strong ammonia and 10 grams of ammonium chlorid and making the whole up to one liter.) The addition of the ammonia dissolves the purin bases, leaving the uric acid in the precipitate. The original mixture is now filtered in such a way that two 100 c.c. portions are obtained, each portion representing 50 c.c. of urine. With the first of these portions an approximate estimation of the uric acid is made, while with the second the accurate one is carried out.

To one of these portions, fiftieth-normal potassium iodid solution is added from a buret, a few drops being removed from time to time and added to a solution of nitrous-sulphuric acid mixed with a little starch paste. (The fiftieth-normal potassium iodid solution should contain 3.32 grams of KI in one liter. The nitrous-sulphuric acid mixture is made by mixing 25 c.c. of H_2SO_4 with 75 c.c. of H_2O and 1 c.c. of fuming HNO_3 .) The addition of the drops of the solution to the indicator is for the purpose of determining the point at which an excess of potassium iodid occurs. This will be shown by the appearance of a distinctly blue contact ring. When the solution shows such a reaction, on adding a few drops of the mixture to the indicator, the number of c.c. of KI is read off, and we are then prepared for the more accurate control. It is wise to keep the indicator cold by immersion in ice-water, as otherwise a violent reaction may occur.

The second 100 c.c. portion is then carefully titrated with the fiftieth-normal KI solution by running directly about 1 c.c. less of this solution into the urine than was required in the first titration. After this point is reached the KI solution should be added drop by drop and a test made after the addition of each five drops. In this way an accurate end-point may be reached. As each 100 c.c. portion represents 50 c.c. of urine it will contain 25 c.c. of fiftieth-normal KI used and the number of c.c. of silver nitrate which combined with the uric acid of the urine is obtained. One c.c. of fiftieth-normal AgNO_3 solution represents 0.00336 gram of uric acid. By multiplying this factor by the number of c.c. of silver nitrate used, we obtain the amount of uric acid in 50 c.c. of urine.

The second part of this method consists in the determination of the total purins. One hundred and ten c.c. of urine are treated with 55 c.c. of fiftieth-normal AgNO_3 solution and diluted with water to 220 c.c. The remainder of the process is exactly as outlined above. The number of c.c. of fiftieth-normal silver nitrate used will represent the values for the total purins.

If the number of c.c. of silver nitrate solution used in the previous determination of uric acid be subtracted from the number used in the determination of total purin, the result will be the number of c.c. referable to the purin

bases. One c.c. of fiftieth-normal AgNO_3 solution represents 0.00152 gram of purin bases calculated as xanthin. By multiplying this factor by the number of c.c. of AgNO_3 used we obtain the amount of xanthin in 50 c.c. of urine.

The writer has used this method frequently and has found its results very similar to those of the Salkowski-Ludwig method, although the amount of uric acid is somewhat less in this method than in the latter one.

It is very simple, is quick and accurate and may, therefore, be recommended for general work. A somewhat similar method is advocated by Bartley.¹ The writer finds this latter method fairly reliable and simple.

Folin and Wu's Colorimetric Method.

This method² combines the use of some of the reagents used in other tests by Folin and his associates together with the introduction of a new permanent standard uric acid solution for comparison in the color tests.

Solutions Necessary.

1. Standard uric acid solution. Before starting to prepare the uric acid solution, a 20 per cent. filtered solution of sodium sulphite should be available. Dissolve 1 gram of pure uric acid in 125 to 150 c.c. of 0.4 per cent. lithium carbonate solution and dilute to a volume of 500 c.c. Transfer 50 c.c., corresponding to 100 mg. of uric acid, to each of a series of volumetric liter flasks. Add about 300 c.c. of water and then add 500 c.c. of clear 20 per cent. sodium sulphite solution, mix, dilute to volume, and mix thoroughly. Fill a series of 200 c.c. bottles with this solution and stopper very tightly in order to reduce the absorption of oxygen from the air.

2. A 10 per cent. sodium sulphite solution, kept like the uric acid solution, in small tightly stoppered bottles.

3. A 5 per cent. solution of sodium cyanide.

4. A solution containing 5 per cent. of silver lactate and 5 per cent. of lactic acid.

5. The uric acid reagent of Folin and Denis.³ This is prepared by boiling 100 grams of sodium tungstate⁴ with 80 c.c. of "syrupy" phosphoric acid (85 per cent.) and 700 c.c. of water for not less than 2 hours (using a reflux condenser) and then diluting to 1 liter. When this reagent reacts with uric acid, a blue solution is formed, which permits of clear-cut color comparisons.

Technic.

Transfer from 1 to 3 c.c. of urine to a 15 c.c. centrifuge tube and mix with enough water to make a volume of about 6 c.c. Add 5 c.c. of the acid silver lactate solution and stir with a fine glass rod (diameter 1 to 2 mm.) Rinse off the rod with a few drops of water and centrifuge. If enough silver

¹ Medical Chemistry, Philadelphia, 1904.

² Jour. Biol. Chem., 1919, XXXVIII, 459. In this connection see Cohentervaert, Dissert., Utrecht, 1917; Jongblved, Pharm. Weekblad., 1920, LVII, 655; Takata, Tohoku Jour. Exp. Med., 1920, I, 460. Benedict and Franke (Jour. Biol. Chem., 1922, LII, 387) have introduced a somewhat similar method employing their arsenic phosphoric tungstic acid reagent.

³ See Folin and Macallum, Ibid., 1912, XIII, 363; Folin and Denis, Ibid., 1913, XIV, 95; Autenrieth and Funk, Münch. med. Wchnschr., 1914, LXI, 457; Höst, Ztschr. f. klin. Med., 1914, LXXXI, 113; Benedict and Hitchcock, Jour. Biol. Chem., 1915, XX, 619.

⁴ Egerer and Ford (Proc. Soc. Exp. Biol. Med., 1918, XVI, 10) call attention to the frequent necessity of purifying the tungstate before use.

solution has been added, the precipitate settles very quickly. Add a drop of silver lactate solution, to insure an excess of this reagent; if a precipitate forms, add more (2 c.c.) and centrifuge again. Pour off the clear supernatant liquid as completely as possible.

To the precipitate in the centrifuge tube add, from a buret, 4 c.c. of 5 per cent. sodium cyanide solution and stir until a perfectly clear solution is obtained. Pour the contents of the tube into a 100 c.c. volumetric flask and rinse the tube and stirring rod, using for this purpose about 15 to 25 c.c. of water. Add 5 c.c. of 10 per cent. sodium sulphite solution (to balance that in the standard uric acid solution). Dilute to a volume of about 50 c.c.

Transfer to another 100 c.c. volumetric flask 5 c.c. of the standard uric acid sulphite solution, containing 0.5 mg. uric acid; add 4 c.c. of the cyanide solution and dilute to about 50 c.c. Then add 20 c.c. of saturated sodium carbonate solution to each flask, mix, and finally add with shaking 2 c.c. of the uric acid reagent. Let stand for 3 to 5 minutes, fill to the mark, mix, and make the color comparison in the usual manner. Artificial light (with "day-lite" glass) is better than day light for this comparison.

To determine the amount of uric acid in milligrams in the volume of urine taken, with the standard set at 20 mm., divide 10 by the reading of the unknown.

Ruhemann's Method.

This method is a very convenient clinical one, although its results are by no means as accurate as those of the preceding methods.¹ What the general practitioner desires, as a rule, is to know whether the uric acid is increased or diminished and does not care as to the absolute value. Such results, giving the total purins, may be obtained for clinical purposes by this method.

It consists in the use of a specially graduated tube, the uricometer, in which are placed the reagents and the urine to be tested (see cut). The calibrations of the tube are such as to represent directly the amount of uric acid in parts per 1000. The principle of the method is the decolorization of an iodine solution by the uric acid of the urine, and the measurement of the amount of urine which must be added to a definite amount of iodine solution to effect this decolorization.

Technic.

Carbon disulphid is placed in the tube up to the mark S, in such a way that the lower meniscus of this reagent rests upon the mark. A solution of iodine in potassium iodid is then added so that the upper portion of the

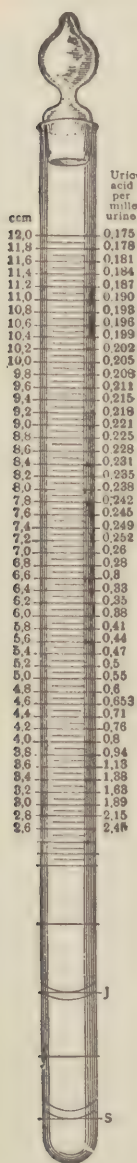


FIG. 79.—Ruhemann's uricometer.

¹ See Bradley and Bunta, Jour. Am. Med. Assn., 1913, LX, 44.

meniscus coincides with the mark J. This iodine solution has the following composition.

Iodin,	0.50 gram
Potassium iodid,	1.25 grams
Absolute alcohol,	7.50 grams
Glycerin,	5.00 grams
Distilled water, q.s.,	100.00 grams

The urine is added slowly by means of a pipet until the lowest calibration is reached. The glass stopper is inserted and the contents of the tube mixed by repeated inversion for about 15 seconds. The carbon disulphid absorbs the iodine, taking on a distinct purple coloration. If this amount of urine does not completely decolorize the iodine, shown by the porcelain-like color of the carbon disulphid solution, more urine is added and the tube again inverted for 15 seconds. This process is continued until repeated shaking of the tube causes the carbon disulphid to assume a pale pink color. The reaction is practically ended at this point, as by a little more shaking of the contents the indicator will assume the characteristic porcelain-white appearance. This process requires from 6 to 15 minutes. The amount of uric acid is then read off directly from the tube in parts per liter.

Should the urine contain less uric acid than can be read off from the calibrations, a second test is made adding the iodine solution to the mark midway between S and J, the amount indicated on the tube being of course divided by 2. Conversely, should the urine contain more uric acid than is represented by the lower calibration, one adds the iodine solution to the point above J and multiplies his reading by 1.5, or adds the iodine solution to the second mark above J and multiplies the reading by 2.

With this method the urine must be acid in reaction. If the urine contains a sediment of the urates, it should be thoroughly shaken before being added, so that the urates may be in suspension. Any free uric acid which may have separated in the sediment is not determined in this method. Strongly colored urines have no influence upon the decolorization. The presence of sugar does not interfere with the results, but if albumin be present in large amounts it should be removed by acidifying with dilute acetic acid, boiling, and filtering. The writer has used this method very frequently and finds it very useful for rough estimation of the uric acid outputs. The purinometer, introduced by Hall,¹ seems to be a much more reliable and useful instrument, as it employs the reagents required by the Salkowski-Ludwig method.

(e) Purin Bases.

These bodies have been called purin bases, alloxur bases, xanthin bases, and nuclein bases. They are of extreme importance from the standpoint of physiologic chemistry, but are, clinically, of less value than is uric acid. The following bodies have been isolated from the urine in various conditions: adenin, guanin, epiguanin, carnin, episarkin, xanthin, hypoxanthin, heter-

¹ The Purin Bodies, Phila., 1904.

oxanthin, paraxanthin, and methylxanthin. Certain methylated xanthin compounds are found in tea and coffee and are, therefore, introduced into the system as caffeine, theobromin, and theophyllin, being excreted either as xanthin or hypoxanthin. Xanthin ($C_5H_4N_4O_2$) is 2, 6, dioxypurin.

These nuclein bases of the urine arise either from the nuclein of the food or from the increased nuclein metabolism of the system. Very little is known at the present time regarding the absolute variations in the excretion of these purin bases in the urine. Salkowski finds an excretion ranging between 0.0286 and 0.0561 gram (calculated as xanthin), while Camerer regards an average output as 0.087 gram in 24 hours. It is interesting to note that a vegetable diet appears to increase this output more than a meat diet, 0.044 gram being excreted, according to Camerer, on a meat diet and 0.111 gram on a vegetable régime. This finding is the reverse of that for uric acid. As a general rule, it may be said that the output of purin bases is from 8 to 10 per cent. of that of uric acid, varying from 16 to 60 mg. per diem. It is evident that foods containing these substances should be absolutely interdicted in conditions which may be traceable to disturbances in the nuclein metabolism.

An increase in the amount of uric acid is usually associated with an increase of the xanthin bases, but at times no such relations obtain, a decrease in these bodies being observed. In leukemia an excretion of 0.321 gram has been reported by Magnus-Levy.¹ In certain cases of tuberculosis, nephritis, epilepsy, migraine, and pneumonia the output may be much increased. Edsall finds the urinary output increased as a result of X-ray treatment. Xanthin is occasionally found as a constituent of the urinary sediment and may form calculi.²

(f) **Creatinin ($C_4H_7N_3O$)**.

The older ideas regarding the excretion of this nitrogenous body have suffered a severe shock from the work principally of Folin, Shaffer, Hoogenhuyze and Verploegh, Mellanby, and Klercker. Acid urines were supposed to contain creatinin and little or no creatin; while alkaline urines were thought to show creatin instead of creatinin. It has been proven, however, that "normal fresh urine, whether acid or alkaline, contains creatinin, and if the normal subject has not taken creatin in his food during the preceding days his urine will not contain creatin, whatever its reaction. There is no normal excretion of endogenous creatin,³ as this, when ingested, is largely retained in the body unless the food contains a large amount of protein." Thus Folin has shown that the excretion of creatinin on a diet yielding a urinary nitrogen value of 16 grams, was 1.55 grams or 3.6 per cent. of total nitrogen; while on a nitrogen-free diet with a urinary nitrogen of 3.6 grams, the creatinin output was 0.6 gram or 17.2 per cent. of total N. He showed, further, that while the actual amount excreted varied with different individuals, yet for the same person the

¹ *Loc. cit.*

² See Heffer, *Deutsch. Arch. f. klin. Med.*, 1913, CIX, 322; also, Koch, *Jour. Biol. Chem.*, 1913, XV, 43.

³ This statement does not apply to growing children who show a normal excretion of a relatively large amount of creatin. See Mendel and Rose, *Jour. Biol. Chem.*, 1911, X, 213, 255 and 265; also Folin and Denis, *Ibid.*, 1912, XI, 253; Schiff and Bálint, *Arch. f. Verd.-Kr.*, 1921 LXIX, 439; Iseke, *Monatssch. f. Kinderhke.*, 1921, XXI, 337.

output was practically constant, under the same conditions of health and muscular activity. He believes, therefore, that creatinin is by far the most reliable index as to the amount of a certain kind of protein metabolism occurring daily in any given individual. He bases his conclusions upon the facts that "the absolute quantity of creatinin eliminated in the urine on a meat-free diet is a constant quantity, differing for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated." Shaffer¹ believes that creatinin is not an index of the total endogenous protein catabolism, as patients in whom the endogenous catabolism is much increased may excrete very little creatinin. Folin finds that the chief factor determining the amount of creatinin eliminated on any special diet is the weight of the patient. Fat or corpulent persons yield less creatinin per unit of body-weight than do lean ones. It is, therefore, necessary in metabolic work to consider not only the body-weight, but, also, the excess of fat in increasing the weight.

According to Shaffer, the normal excretion of creatinin varies between 7 and 11 mg. of creatinin-nitrogen per kilo of body-weight. In pathologic subjects it is low, varying from the normal to 2 mg. per kilo of body-weight in 24 hours. He calls the creatinin-nitrogen excretion per kilo of weight the "creatinin coefficient." He thinks that creatinin is an index of some special process of normal metabolism taking place largely, if not wholly, in the muscles. Upon the intensity of this process appears to depend the muscular efficiency of the individual. In acute febrile conditions, in which an increased destruction of muscle tissue occurs, an increase is seen in the creatinin output during the active febrile period, while in the period of convalescence a diminished excretion will be observed. This excretion in fever does not run parallel to the muscular efficiency of the individual (Shaffer). Simon has shown that a diminished excretion occurs in anemia, marasmus, myositis ossificans, chlorosis, phthisis, chronic parenchymatous nephritis, progressive muscular atrophy, and pseudohypertrophic paralysis.

According to Shaffer, creatin may be excreted by subjects of acute fevers, in the acute stages of exophthalmic goiter, in other conditions in which there is a rapid loss of muscle protein, and by women during the postpartum resolution of the uterus. Krause² has reported the constant presence of creatin in diabetes mellitus referable, probably, to a deprivation of carbohydrates. Although administration of carbohydrate usually diminishes the creatin excretion, Folin's theory would indicate that an increased excretion of creatin may follow a carbohydrate-rich diet, if the state of nutrition of the body is high, as a greater fraction of the creatin of the food would be eliminated. The study of the excretion of creatinin and creatin in various conditions is fast becoming of the greatest importance.³ Infants and children regularly show a creatinuria on normal diets and under normal conditions of health.

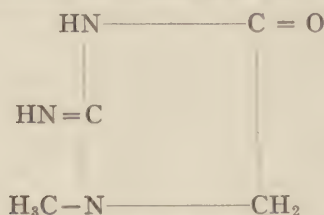
¹ *Am. Jour. Physiol.*, 1908, XXIII, 1.

² *Quart. Jour. Exper. Physiol.*, 1920, III, 289. Gross and Steenbock (*Jour. Biol. Chem.*, 1921, XLVII, 33 and 45) believe that augmented breaking down of protein, whether this be derived from the food or be of endogenous origin, liberates arginin, which is the precursor of creatin. See, Hammett, *Jour. Am. Med. Assoc.*, 1921, LXXVI, 502.

³ See Mendel, *Science*, 1909, XXIX, 584; Ditman and Welker, *New York Med. Jour.*; 1909, LXXXIX, 1000, 1046 and 1091; Ellis, *Jour. Am. Med. Assn.*, 1911, LVI, 1870.

Qualitative Tests for Creatinin.

Creatinin has the formula, $C_4H_7N_3O$, with the graphic structure



The chemical reactions which serve for the detection of creatinin depend upon the formation of different colored compounds. It forms a distinct crystalline compound with zinc chlorid, which may be used in the quantitative estimation by Salkowski's method. For this process see works on physiologic chemistry. A point to be remembered regarding creatinin is that it reduces copper solutions and may be mistaken for sugar unless care be exercised.

Weyl's Test.

To the urine to be tested are added a few drops of a very dilute aqueous solution of freshly dissolved sodium nitroprussid and a few drops of dilute sodium hydrate solution. In the presence of creatinin a ruby-red color appears which changes, after a short time, to an intense yellow. If this solution be heated with a little glacial acetic acid the color will change to green and finally blue.

Skutetzky, Deutsch. Arch. f. klin. Med., 1911, CIII, 423; McCrudden, Jour. Exper. Med., 1912, XV, 457; Sedgwick, Am. Jour. Dis. Child., 1912, III, 209; Kraus, Arch. Int. Med., 1913, XI, 613; Scaffidi, Internat. Beitr. z. Ernährungsstör., 1913, IV, 401; Orioli, Ibid., 421; Mellanby, Proc. Roy. Soc., 1913, LXXXVI, 88; Myers and Volovic, Jour. Biol. Chem., 1913, XIV, 489; Myers and Fine, Ibid., 1913, XV, 283 and 305; Ibid., 1913, XVI, 169; Thomas and Goerne, Ztschr. f. physiol. Chem., 1914, XCII, 163; Lampert, Ztschr. f. klin. Med., 1914, LXXX, 498; Folin and Morris, Jour. Biol. Chem., 1914, XVII, 469; Folin and Denis, Ibid., 493; Benedict, Ibid., 1914, XVIII, 183 and 191; Benedict and Osterberg, Ibid., 195; Shaffer, Ibid., 525; Tracy and Clark, Ibid., 1914, XIX, 115; Palmer, Means and Gamble, Ibid., 239; Ringer and Raiziss, Ibid., 487; Hull, Jour. Am. Chem. Soc., 1914, XXXVI, 2146; Morris, Jour. Biol. Chem., 1915, XXI, 201; Myers and Fine, Ibid., 377, 383 and 389; Janney and Blatherwick, Ibid., 567; Myers and Fine, Ibid., 583; Taylor, Ibid., 663; Baumann and Marker, Ibid., 1915, XXII, 49; MacAdam, Biochem. Jour., 1915, IX, 229; Practitioner, 1914, XCII, 540; Hutchison, Indian Jour. Med. Research, 1915, II, 814; van Hoogenhuyze, Nederl. Tijdschr. v. Geneesk., 1915, I, 1786; Detweiler and Griffith, New York Med. Jour., 1915, CII, 798; Morse, Jour. A. M. A., 1915, LXV, 1613; Tsuji, Biochem. Jour., 1915, IX, 449; Baumann and Marker, Jour. Biol. Chem., 1915, XXII, 49; Rose, Ibid., 1916, XXVI, 331; Rose, Dimmitt and Cheatham, Ibid., 330; Rose and Dimmitt, Ibid., 345; Denis, Ibid., 379; Underhill, Ibid., 1916, XXVII, 127 and 141; Underhill and Baumann, Ibid., 147, 151 and 160; Underhill and Bogert, Ibid., 161; Burns and Orr, Biochem. Jour., 1916, X, 495; Powis and Raper, Ibid., 363; Cutter and Morse, Am. Jour. Dis. Child., 1916, XI, 326 and 331; Veeder and Johnston, Ibid., 1916, XII, 136; Lyman and Trimby, Jour. Biol. Chem., 1917, XXIX, 1; Denis, Ibid., 447; Ibid., 1917, XXX, 47; Denis and Kramer, Ibid., 189; Baumann and Hines, Ibid., 1917, XXXI, 549; Denis and Minot, Ibid., 561; Rose, Ibid., 1917, XXXII, 1; Hunter and Campbell, Ibid., 1918, XXXIV, 5; Rose, Dimmitt and Bartlett, Ibid., 601; Baumann and Hines, Ibid., 1918, XXXV, 75; Baumann and Ingvaldsen, Ibid., 277; Steenbock and Gross, Ibid., 1918, XXXVI, 265; McClure, Arch. Int. Med., 1918, XXII, 719; Denis and Minot, Jour. Biol. Chem. 1919 XXXVII, 245; Gamble and Goldschmidt, Ibid. XL, 199 and 215; Beeker, Ned. Tijdschr. v. Geneesk., 1919, I, 431; Ziegler and Pearce, Jour. Biol. Chem., 1920, XLII, 581; Hahn and Barkan, Ztschr. f. Biol., 1920, LXXII, 25; Meyer, Deutsch. Arch. f. klin. Med., 1920, CXXXIV, 219; Pemberton and Buckman, Arch. Int. Med., 1920, XXV, 335; Raphael and Eldridge, Ibid., 1921, XXVII, 604; Hammett, Jour. Biol. Chem., 1921, XLVIII, 127 and 133; Gibson and Martin, Ibid., 1921, XLIX, 319; Weinberg, Biochem. Jour., 1921, XV, 306; Schultz, Arch. f. ges. Physiol., 1921, CLXXXVI, 726; Gibson, Martin and Buell, Arch. Int. Med., 1922, XXIX, 82; Mitchell, Neveus and Kendall, Jour. Biol. Chem., 1922, LII, 417.

Acetone gives a similar reaction, but on the addition of acetic acid changes to a purplish-red instead of green. If the urine be heated previous to the application of this test, the acetone may be driven off. This test for creatinin is sensitive to 6 parts in 10,000.

Jaffé's Test.

To the urine to be tested are added a few drops of a saturated solution of picric acid and a few drops of dilute sodium hydrate solution. If creatinin be present a red color appears immediately, which increases in intensity and remains permanent for a long time. If glacial acetic acid be added the color becomes yellow. Acetone gives a reddish-yellow color of less intensity than that produced by creatinin. Glucose, if present, may give a red color if the mixture be warmed. This test indicates one part of creatinin in 5,000.

Quantitative Determination.

Folin's Older Method.

The principle upon which this determination¹ is based is the comparison of the color produced by Jaffé's reaction with that of a standard solution of potassium bichromate. A high-grade colorimeter is necessary for this comparison. Folin recommends the use of the Duboscq instrument, while the writer finds one made by Sargent & Co., of Chicago, very satisfactory.² This latter has the advantage of being much less expensive.

The reagents necessary are (1) a half-normal potassium bichromate solution containing 24.55 grams per liter, (2) a saturated picric acid solution containing about 12 grams per liter,³ and (3) a 10 per cent. solution of sodium hydrate.

Technic.

Ten c.c. of urine are measured into a 500 c.c. volumetric flask, 15 c.c. of the picric acid solution and 5 c.c. of the sodium hydrate solution are then

¹ Am. Jour. Physiol., 1905, XIII, 45.

² See Kober, Jour. Biol. Chem., 1917, XXIX, 155; Bock and Benedict, *Ibid.*, 1918, XXXV, 227.

³ As it had been shown by McCrudden and Sargent (Jour. Biol. Chem., 1916, XXIV, 423 and 527) as well as by Hunter and Campbell (*Ibid.*, 1917, XXVIII, 335) that serious errors and discrepancies were obtained by the use of Folin's method; Folin and Doisy (*Ibid.*, 349) found that the trouble was due to impurities in the picric acid as obtainable on the market. For this reason the picric acid, used in determinations of creatinin, should either be known to be pure when purchased or should be purified by the following method. Transfer about 600 grams of wet picric acid, or about a pound of dry picric acid, to a large beaker (capacity not less than 4 liters). Pour on boiling water until the beaker is nearly full and add 200 c.c. of saturated (50 per cent.) sodium hydrate solution. Stir, and if necessary heat again until all the picric acid has dissolved, yielding a deep red picrate solution. To the hot solution add, rather slowly, with stirring, 200 grams of sodium chlorid. Cool in running water to about 30°C., with occasional stirring. Filter on a large Buchner funnel and wash a few times with 5 per cent. sodium chlorid solution. Transfer the picrate to a large beaker, fill with boiling water, and when the picrate is dissolved add, with stirring, first 50 c.c. of 10 per cent. sodium hydrate solution, and then 100 grams of sodium chlorid. Cool to 30°C., with stirring, filter, and wash with sodium chloride solution, as before. Repeat the solution and precipitation of the sodium picrate twice more, but for the last washing of the last precipitated picrate use distilled water instead of sodium chlorid solution. Dissolve the purified picrate in the same large beaker, with boiling distilled water, and filter hot on a large folded filter, collecting the filtrate in a large flask. To the hot filtrate add 100 c.c. of concentrated sulphuric acid, previously diluted with about two volumes of water. The liberated picric acid begins to come out at once. Put a beaker over the mouth of the flask and cool under running tap water to about 30°C. Filter with suction and wash free from sulphates with distilled water.

added, and the mixture allowed to stand for five or six minutes. This interval is used to pour a little of the standard bichromate solution into each of the two cylinders of the colorimeter. The depth of the solution in one of the cylinders is then accurately adjusted to the 8 mm. mark. With the solution in the other cylinder a few preliminary colorimetric readings are made simply for the sake of insuring greater accuracy in the subsequent readings of the unknown solution. The two bichromate solutions must, of course, be equal in color, and in

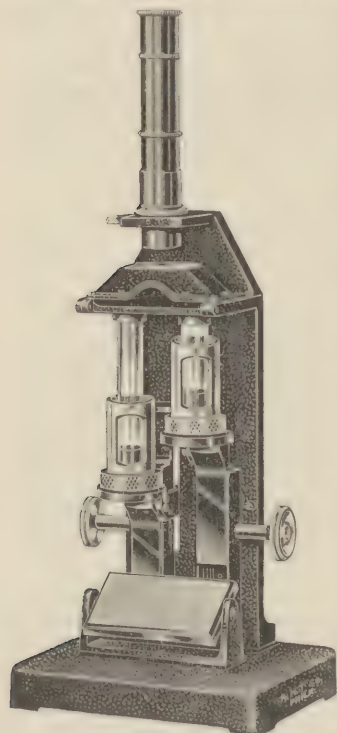


FIG. 80.—Duboscq Colorimeter.

taking their readings no two should differ more than 0.1 mm. or 0.2 mm. from the true value (8 mm.), leaving out of consideration the very first reading made, which is sometimes less accurate. Four or more readings should be made in each case, and an average taken of all but the first. After a while one becomes sure of the true point, and can take the average of the first two readings.

At the end of five minutes the contents in the 500 c.c. flask are diluted up to the 500 c.c. mark. The bichromate solution is thoroughly rinsed out of one of the cylinders by means of the unknown solution and several colorimetric readings are then made *at once*. The calculation of the results is very simple. It is based on the experimentally determined fact that 10 mg. of perfectly pure creatinin give, under the conditions of the determination, 500 c.c. of a solution, 8.1 mm. of which have exactly the same colorimetric value as 8 mm. of a half-normal bichromate solution.¹

If, for example, it is found that it takes 9.5 mm. of the unknown urine picrate solution to equal the 8 mm. of the bichromate, then the 10 c.c. of urine contain

$$10 \times \frac{8.1}{9.5} = 8.4 + \text{mg. of creatinin.}$$

If the 10 c.c. of urine used in the test are found to contain more than 15 mg. or less than 5 mg. of creatinin, the determination should be repeated with a correspondingly different amount of urine, because outside of these limits the determination is much less accurate. The color of the urine does not materially affect the results on account of the great dilution. Sugar and albu-

¹ This relationship applies to the Duboscq instrument, which Folin advises. If one uses the Sargent or other colorimeter, he must, of course, make his adjustment according to his special instrument. It matters little what point is used for the standard solution, providing one has tested his bichromate solution against solutions of creatinin treated as above and has obtained his relative values.

min do not interfere with the determination, while acetone and diacetic acid do unless one allows a few minutes before reading.¹

Folin's Newer Method.

In order to enlarge the scope of the colorimetric method for the determination of creatinin and creatin, Folin advises the replacing of the bichromate solution with a standard creatinin solution for the color comparison. While creatinin may be obtained on the market it is usually far from pure, so that one should prepare his own creatinin according to the method of Folin² or of Benedict.³

The method is as follows: One c.c. of a standard solution of creatinin (1 gram per liter, hence 1 c.c. containing 1 mg.)⁴ is measured into a 100 c.c. volumetric flask and 1 c.c. of the urine into a similar flask by means of the accurate Ostwald pipets (care being taken to allow the pipet to drain against the neck of the flask for a few seconds and then to blow out the pipet clean). Twenty c.c. of saturated picric acid solution (measured with a cylinder) are added to each flask and then 1.5 c.c. of 10 per cent. solution of sodium hydrate are introduced from a buret. At the end of 10 minutes the flasks are filled up to the mark with tap water, are mixed thoroughly and the relative color of the two solutions determined in the colorimeter. It makes little difference whether the standard solution is set at 10, 15 or 20 mm., as the comparisons are equally exact at any one of these points. This standard reading is divided by the reading of the unknown to obtain the milligrams of creatinin present in the volume of urine (1 c.c.) taken. If the urine reads less than two-thirds or more than one and one-half that of the standard the determination should be repeated with more or less urine as the case may be.

Creatin.

The determination of creatin, as well as of creatinin, is of much importance in various conditions. Many methods have been given for such work but the following seems to offer the best results with the simplest conditions.

Folin's Method.⁵

Enough urine to give 0.7 to 1.5 mg. of creatinin is measured into a weighed Erlenmeyer Jena flask, (capacity 200 c.c.). Twenty c.c. of a saturated picric solution, about 130 c.c. of water and a few very small beads to promote even boiling are added and the mixture is gently boiled, preferably over a microburner, for about 1 hour. At the end of this time increase the heat and boil down the solution to about 20 c.c. Transfer the flask to the scales and add enough water to make the total solution equal to 20 to 25 grams. Cool the solution in running water, add 1.5 c.c. of 10 per cent. sodium hydrate

¹ See Greenwald, Jour. Biol. Chem., 1913, XIV, 87; also, Thompson, Wallace and Clotworthy, Biochem. Jour., 1913, VII, 445. See Dehn, Jour. Am. Chem. Soc., 1917, XXXIX, 1302 for possible errors in this test; also, Allen and Davisson, Jour. Biol. Chem., 1919, XL, 183. Blau (Jour. Biol. Chem., 1921, XLVIII, 105) recommends boiling 10 c.c. of urine in a 300 c.c. flask with 5 volumes of methyl alcohol in order to remove the interfering acetone and diacetic acid.

² Jour. Biol. Chem., 1914, XVII, 463 and 469.

³ Jour. Biol. Chem., 1914, XVIII, 183.

⁴ Instead of the creatinin itself, one may use the creatinin-zinc chlorid dissolving 1.6106 grams in a liter of N/10 hydrochloric acid. This salt is more easily prepared in a pure state than is the creatinin.

⁵ Jour. Biol. Chem., 1914, XVII, 469. See, also, Benedict, Ibid., 1914, XVIII, 191.

solution and determine the total creatinin as in the above method, using 1 mg. of pure creatinin solution as a standard.

This method gives both the preformed creatinin and that derived from the creatin. Subtract the preformed (as determined by the direct method) from the total creatinin and obtain the creatinin derived from creatin. Multiply this latter figure by 1.16 to determine the actual amount of creatin as such.

(g) Undetermined Nitrogen.

By the undetermined nitrogen of the urine is meant the nitrogen remaining after that attributable to urea, uric acid, xanthin bases, ammonia, and creatinin has been subtracted from the total nitrogenous output. This factor is made up of many substances present in variable amount and determined with more or less difficulty.¹

As the variations in the other nitrogenous constituents have been shown to depend to a great extent upon the nature of the diet, so we would expect the values for the undetermined nitrogen to show similar fluctuations. Folin finds on a diet yielding 16 grams of total nitrogen in the urine an undetermined nitrogen output of 0.6 gram or 3.75 per cent. of total nitrogen; while on a nitrogen-free diet with a urinary nitrogen value of 3.6 grams the undetermined nitrogen is 0.27 gram or 7.3 per cent. He is led, therefore, to make the following generalization: "The absolute quantity of undetermined nitrogen decreases under the influence of the starch and cream diet, but in per cent. of the total nitrogen there is always an increase." The separation of the various factors included in this undetermined nitrogen has clinically little value at the present time.

(1) Amino Acids.

Theoretically, we should find in the urine, whenever hepatic metabolism is disturbed, both mono- and di-amino acids, as these substances are normal products of protein hydrolysis in the intestine and are directly converted into urea in the normal liver. It has long been known that acute yellow atrophy and phosphorus poisoning were associated with a diminished urea output and the presence of the two mono-amino acids, leucin and tyrosin, in the urine. Recent work² with improved methods has shown that the urine contains these and other amino acids in any condition in which hepatic activity is impaired, so that our old-time diagnostic point of acute yellow atrophy must fall by the wayside. Although these substances are found in much larger quantities under pathologic conditions, traces of them are present in normal urine,

¹ Webster, Brit. Med. Jour., 1916, II, 845, has advanced a method for detection of small amounts of certain organic nitro compounds in the urine of TNT workers. See, also Elvove, Jour. Indus. and Eng. Chem., 1919, XI, 860.

² See Signorelli, Biochem. Ztschr., 1912, XXXIX, 36; Ibid., 1912, XLVII, 482; Galambos and Tausz, Ztschr. f. klin. Med., 1913, LXXVII, 14; Damask, Ztschr. f. klin. Med., 1913, LXXVII, 333; Hugounenq, Presse méd., 1913, XXI, 825; Van Slyke and Meyer, Jour. Biol. Chem., 1913, XVI, 213; and Löffler, Ztschr. f. klin. Med., 1913, LXXVIII, 483; Benedict and Murlin, Jour. Biol. Chem., 1913, XVI, 385; Labbé and Bith, Rev. de méd., 1914, XXXIV, 89; Udaondo and Casteigts, Sem. med., 1914, XXI, 741; Geake and Nierenstein, Ztschr. f. physiol. Chem., 1914, XCII, 149; Folin, Jour. Am. Med. Assn., 1914, LXIII, 823; Harding and MacLean, Jour. Biol. Chem., 1915, XX, 217; Van Slyke, Ibid., XXIII, 407; Bang, Biochem. Ztschr., 1916, LXXII, 101; Harding and MacLean, Jour. Biol. Chem., 1916, XXIV, 503; Van Slyke, Arch. Int. Med., 1917, XIX, 56; De Graaff and van der Zande, Pharm. Weekblad, 1916, LIII, 1378; Shiple and Sherwin, Jour. Am. Chem. Soc., 1922, XLIV, 618; Folin and Berglund, Jour. Biol. Chem., 1922, LI, 395.

especially when the nitrogen intake has been large. Thus von Noorden regards glycocoll (amino-acetic acid) as a normal urinary constituent, its excretion averaging 1 gram daily. These acids are also found in cases of gout, pneumonia, especially during the absorption of the exudate, in diabetes, and in leukemia.¹

A large number of these mon-amino acids have been isolated from the direct products of protein hydrolysis, but the number found in the urine has not been as large owing to the uncertain methods of examination. The introduction by Fischer of the esterification method has added much to our knowledge of these bodies. By the di-amino acids we mean the bodies lysin,² arginin and histidin, which are collectively known as the hexone bases.

Benedict and Murlin's Method.

This method³ is not as accurate as the gasometric one of Van Slyke,⁴ but is much more suitable for clinical work.

Measure into a 500 c.c. Erlenmeyer flask 200 c.c. of a 24-hour specimen of urine, which has been diluted to 2,000 c.c. Add an equal quantity of a 10 per cent. solution of Merck's phosphotungstic acid in 2 per cent. hydrochloric acid. Let the mixture stand at least 3 hours, preferably over night. Pour off 250 c.c. of the clear supernatant fluid, add 1 c.c. of a 0.5 per cent. alcoholic solution of phenolphthalein and then barium hydrate in substance until the whole fluid turns decidedly pink (this latter substance should be added a little at a time to avoid an excess). Let this mixture stand for 1 hour. Now filter off two 100 c.c. samples, each representing 50 c.c. of urine. Neutralize these specimens to litmus paper by means of N/5 hydrochloric acid and add at once, to each, 10 to 20 c.c. of formalin which has been previously neutralized with N/10 sodium hydrate solution. Titrate the mixture cautiously with N/10 NaOH to a deep red color, *i.e.*, until the drop of alkali produces no additional color. Make the duplicate determination in the same way and correct the results by deduction of the amount of N/10 NaOH necessary to produce the same depth of color in an equal quantity of CO₂-free water with the same quantity of neutral forma-

¹ Warfield (Jour. Am. Med. Assn., 1914, LXII, 436) has attempted to base a diagnostic test for pregnancy on the presence in the urine of dialyzable products reacting to ninhydrin. This test, however, has no value as the urine, normal or abnormal, always contains dialyzable substances, especially amino acids and certain ammonium compounds, which under proper conditions will react to ninhydrin. In this connection see Abderhalden's test in the section on Blood; also, Jamison, Jour. Am. Med. Assn., 1914, LXII, 1084; Fischer, *Ibid.*, 950 and 1575; Falls and Welker, *Ibid.*, 1800; Berman, Am. Jour. Obs., 1914, LXX, 192; Holmes, Lancet-Clinic, 1914, CXI, 400.

Malone (Jour. Am. Med. Assn., 1915, LXIV, 1651), using a modification of the technic of Kiatsi (Urine Diagnosis by Means of Filtration Process, Sapporo, Japan, 1914), removes the biuret-reacting urinary substances by means of the addition of 0.3 gram of kaolin to 15 c.c. of urine, filters this mixture and neutralizes the filtrate. This is then submitted to the usual Abderhalden technic, employing 10 c.c. of the biuret-free filtrate and 0.2 gram of dried placenta. This latter test may prove to be of some diagnostic value. See Cutler and Morse, Jour. A. M. A., 1916, LXVI, 559; Berkowitz, Arch. Int. Med., 1917, XIX, 397.

² See Ackermann and Kutscher (Ztschr. f. Biol., 1911, LVII, 355) who report the presence of lysin in a patient with cystinuria. Kocher (Jour. Biol. Chem., 1915, XXII, 295) finds these diamino acids largely increased in malignant tissues. Edlbacher, Ztschr. f. physiol. chem., 1915, XCV, 81.

³ Jour. Biol. Chem., 1913, XVI, 385.

⁴ *Ibid.*, 121 and 125. See, also, Chodat and Krummer, Biochem. Ztschr., 1914, LXV, 392; Harding and MacLean, Jour. Biol. Chem., 1915, XX, 217; Willard and Cake, Jour. Am. Chem. Soc., 1920, XLII, 2646; Foreman, Biochem. Jour. 1920, XIV, 451; Ciaccio, Arch. sci. med., 1920, XLIII, 177; Mestrezat, Medicine, 1921, II, 970.

lin added. Each c.c. of $N/10$ NaOH used in the titration, corrected as above, represents 0.0014 gram of nitrogen referable to the amino acids. Multiply the nitrogen value by 2 to obtain the percentage, and this latter amount by 20 to derive the total amino-acid nitrogen in the 24-hour specimen.

Folin's Method.

This method¹ is similar to the one for the amino-acid nitrogen of the blood, but with the urine the ammonia must first be removed.

Dilute from 5 to 25 c.c. of urine to a volume of 25 c.c. in a 50 c.c. Erlenmeyer flask. Add 2 to 3 grams of permittit and agitate very gently, but continuously, for 5 minutes. Decant the supernatant urine into another 50 c.c. flask. Again add 2 to 3 grams of permittit, and shake as before for 5 minutes. By this double extraction with permittit every trace of ammonia is removed. Decant the supernatant urine into a flask or test-tube. If this be a little turbid it does not interfere with the determination.

To test-tubes graduated at 25 c.c. add 1, 2, and 3 c.c., respectively, of a standard solution of glycooll (glycin) in 0.1 N hydrochloric acid plus 0.2 per cent. of sodium benzoate. This standard should contain 0.1 mg. of glycooll nitrogen per c.c. Dissolve for this purpose 53.5 mg. of glycooll in 100 c.c. of the above menstruum. To these tubes, containing the standard solution, add 1, 2, and 3 c.c., respectively, of the special 1 per cent. sodium carbonate solution.² Dilute the contents of each tube to a volume of 10 c.c.

Transfer 5 c.c. of the ammonia-free (usually diluted) urine to another test-tube graduated at 25 c.c. Add 1 c.c. of 0.1 N hydrochloric acid and 1 c.c. of the 1 per cent. sodium carbonate solution. Dilute to 10 c.c. Dissolve 250 mg. of the amino-acid reagent³ in 50 c.c. of water, and add 5 c.c. of this solution to each standard and to the unknown urine. Mix and set in a dark place over night. It is often advisable to take out the test-tubes and inspect them after they have stood 10 to 15 minutes. If the test-tube containing the urine appears much darker than the darkest standard, as may happen, it is necessary to start another sample of urine, taking only 1, 2, or 3 c.c. and treating it in the same way as the first sample, not omitting to provide for a final volume of 15 c.c.

The following day the standard and the unknown are first acidified by the addition of 1 c.c. of the special 25 per cent. acetic acid-acetate solution.⁴ To each are then added 5 c.c. of the 4 per cent. sodium thiosulphate solution.⁵ The contents of all the tubes are diluted to a volume of 25 c.c. and, after

¹ Jour. Biol. Chem., 1922, LI, 393.

² 50 c.c. of approximately saturated solution (22 per cent.) of sodium carbonate are diluted to a volume of 500 c.c. The strength of the resulting solution is determined by titrating 20 c.c. of 0.1 N hydrochloric acid with the carbonate solution, using methyl red as an indicator. On the basis of the titration value thus obtained, the carbonate solution is diluted so that 8.5 c.c. are equivalent to 20 c.c. of the 0.1 N acid.

³ This is a fresh 0.5 per cent. aqueous solution of the sodium salt of (β -Naphthoquinone-sulphonic acid. Folin (Jour. Biol. Chem., 1922, LI, 386) gives the method for the preparation of this quinone in pure form.

⁴ Dilute 100 c.c. of 50 per cent. acetic acid with an equal volume of 5 per cent. sodium acetate solution.

⁵ This is made in the usual manner from sodium thiosulphate ($Na_2S_2O_3 \cdot 5H_2O$), by dissolving 4 grams in 100 c.c. of water. It is used to destroy the surplus quinone remaining after the full color obtainable from the amino-acids has developed.

mixing, the color of the unknown is read against that of the standard having most nearly the same intensity of color. For the calculation, it is of course, necessary to know which standard is used and the actual volume of undiluted urine taken for the determination.

(2) Hippuric Acid ($C_9H_9NO_3$).

This acid is a normal constituent of urine and varies between 0.1 and 1 gram in 24 hours. It is derived to a large extent from foods containing benzoic acid, such as prunes, cranberries, green-gages, bilberries and many other fruits, and is also formed from the metabolism of tissue and food protein. This substance is directly synthesized in the system by the combination of benzoic acid with glycocoll, but the exact place of synthesis is uncertain. A pathologic increase in the excretion of hippuric acid has been observed in acute febrile diseases, marked intestinal putrefaction, hepatic disturbances, and in diabetes mellitus, while in cases of acute diffuse and chronic parenchymatous nephritis as well as in amyloid kidneys hippuric acid is practically absent from the urine.¹

(3) Oxyproteic and Alloxyproteic Acids.

These acids have been isolated from the urine and seem to be constant constituents, derived from protein catabolism. Both of them contain sulphur and have been credited with forming a large percentage of the neutral sulphur output as well as contributing 2 to 3 per cent. of the total N excretion.² Bondzynski believes the oxyproteic acid accountable for Ehrlich's diazo-reaction (page 368). Salkowski³ has shown that a certain amount (3 to 4 per cent.) of the urinary nitrogen is precipitable by absolute alcohol and is non-dialyzable. This is the so-called "*Colloidal N*" and is largely referable to oxyproteic acid and N-containing carbohydrates. As this factor is increased in many cases of carcinoma up to 9 or 10 per cent. of total N, he believes this may be of value in diagnosis. However, numerous workers have demonstrated that this increase does not always obtain in cases of carcinoma and is found in pregnancy, diabetes, some hepatic disorders and in pulmonary tuberculosis. Salmon and Saxl have outlined a method for the determination of the sulphur factor of this oxyproteic acid as related to total N, and believe that an increase in this factor is indicative of carcinoma.⁴

¹ Dakin, Jour. Biol. Chem., 1910, VII, 103; Steenbock, Ibid., 1912, XI, 201; Folin and Flanders, Ibid., 257; Hryntschak, Biochem. Ztschr., 1912, XLIII, 315; Raiziss and Dubin, Jour. Biol. Chem., 1915, XX, 125; Lackner, Levinson and Morse, Biochem. Jour., 1918, XII, 184; Filippi, Arch. farm. sper., 1918, XXVI, 243; Kingsbury and Swanson, Jour. Biol. Chem., 1921, XLVIII, 13; Arch. Int. Med., 1921, XXVIII, 220; Snapper, Nederl. Tijdschr. v. Geneesk., 1921, I, 3044; Ibid., II, 505; Delprat and Whipple, Jour. Biol. Chem., 1921, XLIX, 229.

² See Browinski and Dombrowski, Ztschr. f. physiol. Chem., 1912, LXXVII, 92; Domansky, Monatsschr. f. Psych. u. Neurol., 1912, XXXI, 53; Erben, Prager med. Wchnschr., 1912, XXXVII, 427.

³ Berl. klin. Wchnschr., 1905, XLII, 1581 and 1618; Ibid., 1910, XLVII, 533, 1746 and 2297.

⁴ Marriott and Wolf, Am. Jour. Med. Sc., 1907, CXXXIII, 404; Mancini, Deutsch. Arch. f. klin. Med., 1911, CIII, 288; Caforio, Berl. klin. Wchnschr., 1911, XLVIII, 1843; Kojo, Ztschr. f. physiol. Chem., 1911, LXXIII, 416; Salomon and Saxl, Wien. klin. Wchnschr., 1911, XXIV, 449; Riforma Med., 1911, XXVII, 421; Deutsch. med. Wchnschr., 1912, XXXVIII, 58; Neuberg, Der Harn, 1911, 788; Semenow, Folia Urol., 1912, VII, 215; Berl.

(4) Allantoin ($C_4H_6N_4O_3$).

This substance is a product of oxidation of uric acid. It is somewhat variable in amount, usually being found only in traces, but after a large intake of meat especially rich in nuclein the amount may be quite perceptible. It is found in fairly large quantities in the urine of the new-born, in the amniotic fluid, in ascitic fluid, in traces in the urine of pregnancy, and in leukemic blood. Pathologic variations have been little studied.

The best method for the determination of this substance is that of Wiechowski.¹ A point to be remembered in connection with allantoin is that it may be present in the urine in sufficient quantities to reduce copper solutions and must, therefore, not be mistaken for sugar.

(2) Fatty Acids.

Traces of volatile fatty acids are present in all normal urines. The most important of these are formic,² acetic, propionic, and butyric acids. They are doubtless formed in the intestinal tract by bacterial action upon the carbohydrates of the food, and may, therefore, be to some extent an index of the degree of carbohydrate fermentation. Their normal amount does not much exceed 0.01 gram in 24 hours, although Blumenthal³ gives the figures under an ordinary diet as being equivalent to from 50 to 80 c.c. of tenth-normal sulphuric acid (0.25 to 0.39 gram).

These acids are increased in febrile conditions, the amount running parallel to the rise in temperature. This increase in the fatty acids of the urine is known as *lipaciduria*. According to Rosenfeld, this increase in febrile states is observed only in those cases in which absorption of decomposing albuminous material occurs, as in all suppurative processes within the system. In the convalescent stage of pneumonia these acids may be excreted in increased amount, while a diminished output is usually observed preceding the crisis. In hyperacidity of the gastric contents the fatty acids of the urine are

klin. Wchnschr., 1913, L, 1436; Greenwald, Arch. Int. Med., 1913, XII, 283; Pribram, Münch. med. Wchnschr., 1913, LX, 2047; Dozzi, Gazz. d. osp., 1913, XXXIV, 1007; Marenduzzo, Riforma Med., 1913, XXIX, 1149; Thar and Beneslawski, Biochem. Ztschr., 1913, LII, 435; Lehmann, Deutsch. Arch. f. klin. Med., 1913, CXII, 376; Stadtmüller and Rosenbloom, Arch. Int. Med., 1913, XII, 276; Sassa, Biochem. Ztschr., 1914, LXIV, 105; de Bloeme, Swart and Terwen, Münch. med. Wchnschr., 1914, LXI, 1718; Biochem. Ztschr., 1914, LXV, 345; von Fürth, Ibid., 1915, LXIX, 448; Goodridge and Kahn, Biochem. Bull., 1915, IV, 118; Damask, Wien. klin. Wchnschr., 1915, XXVIII, 499; Goodridge and Kahn, Biochem. Bull., 1915, IV, 118.

¹ Beitr. z. chem. Physiol. u. Path., 1908, XI, 129; Biochem. Ztschr., 1909, XIX, 378; Ibid., 1910, XXV, 431. See, also, Plimmer and Skelton, Biochem. Jour., 1914, VIII, 641; Givens, Jour. Biol. Chem., 1914, XVIII, 417; Pepper and Grier, Jour. Infect. Dis., 1916, XIX, 694; Harding and Young, Jour. Biol. Chem., 1919, XL, 227.

² See Dakin and Wakeman, Jour. Biol. Chem., 1911, IX, 329; Steppuhn and Schellback, Ztschr. f. physiol. Chem., 1912, LXXX, 274; Dakin, Janney and Wakeman, Jour. Biol. Chem., 1913, XIV, 341; and Srisower, Biochem. Ztschr., 1913, LIV, 189, for a discussion of formic acid as an intermediary metabolic product of both carbohydrates and protein. See McNeal and Eldridge, Am. Jour. Dis. Child., 1922, XXIII, 419.

³ Pathologie des Harnes, Berlin, 1903. It has long been known that one of the products of the metabolism of methyl alcohol is formic acid. The excretion of this acid, therefore, might become an indicator of the fact that methyl alcohol had been taken into the body. However, formic acid occurs in the urine of supposedly normal individuals, so that a quantitative determination of this acid is necessary before definite conclusions may be reached. Autenrieth (Münch. med. Wchnschr., 1910, LXVI, 862; Arch. der Pharm., 1920, CCLVIII, 15) has shown that the daily output may reach 0.25 gram. See, also, Stepp, Ztschr. f. physiol. Chem., 1920, CIX, 99.

increased, while in hypoacidity they are diminished. In contradistinction to the increase of fatty acids in cases of fever associated with suppurative conditions, we find a diminution in the amount of fatty acid in scarlet-fever, erysipelas, measles, and diphtheria. In cases of acute rheumatism formic acid is said to be excreted in large amounts. In cyclic vomiting lactic acid¹ may be increased.

A simple method of determining the fatty acids of the urine is to acidify from 250 to 500 c.c. of urine with 50 to 75 c.c. of dilute sulphuric acid and distill. The distillate is then titrated with tenth-normal sodium hydrate solution using phenolphthalein as an indicator. The results are expressed in terms of the corresponding number of c.c. of tenth-normal acid.

(3) Oxalic Acid.

The amount of oxalic acid eliminated in the urine in 24 hours varies from 10 to 20 milligrams. A portion of this excretion is undoubtedly derived from the diet, but some of it is produced in the metabolism of the tissues, especially of those containing nuclein substances. Although the carbohydrates do not take part in the production of oxalic acid normally, we find, in conditions associated with fermentation of the carbohydrates in the stomach, a large increase in the output of oxalic acid. As oxalic acid is more or less readily formed by oxidation of uric acid, passing through the intermediate stage of *oxaluric* acid, we can readily see that increase of nuclein metabolism may increase the output of this substance. Strangely enough, it has been found that the intake either of pure nuclein or of nuclein-containing foods was not associated with a corresponding increase in the urinary oxalic acid, so that we must assume that a large portion of this intake escapes in the feces in the form of calcium oxalate. It has, moreover, been found that the administration of oxalates by the mouth is not associated with increased excretion either in the urine or feces, so that we must assume a decomposition of the oxalic acid into carbon dioxid and water somewhere in the system. This conversion appears to take place in the intestinal canal under the influence of bacterial action. Crystals of calcium oxalate are frequently found in the urine in cases showing a marked increase in the output of ethereal sulphates, so that we must either assume an increased formation from the carbohydrates of the food, an increased intake and absorption of oxalates of the food, or an increase in the nuclein metabolism as a result of absorption of the toxic products of intestinal putrefaction.²

Among the foods which are known to contain relatively large amounts of oxalic acid we find spinach, rhubarb, tomatoes, carrots, celery, string-beans,

¹ For methods of determination see von Fürth and Charnass, *Biochem. Ztschr.*, 1910, XXVI, 199; Mondschein, *Ibid.*, 1912, XLII, 91 and 105; also, Ishihara, *Ibid.*, 1913, L, 468; Levene and Meyer (*Jour. Biol. Chem.*, 1913, XV, 65) have shown that lactic acid is a product of the intermediary metabolism of carbohydrates. See, also, Yoshikawa, *Ztschr. f. physiol. Chem.*, 1913, LXXXVII, 382; von Fürth, *Biochem. Ztschr.*, 1914, XLIV, 131; *Ibid.*, 156; *Wien. klin. Wchnschr.*, 1914, XXVII, 877; Underhill and Steele, *Am. Jour. Dis. Child.*, 1914, VIII, 127; Schneyer, *Biochem. Ztschr.*, 1915, LXX, 294; Maver, *Jour. Biol. Chem.*, 1917, XXXII, 71; Desgrez and Polonowski, *C. R. Acad. des Sc.*, 1920, CLXX, 1008; Polonowski, *C. R. soc. de biol.*, 1920, LXXXIII, 475.

² See Wegrzynowski, *Ztschr. f. physiol. Chem.*, 1913, LXXXIII, 112; also, Aron and Franz, *Monatsschr. f. Kinderh.*, 1914, XII, 645.

green peas, potato, figs, plums, strawberries, pepper, cocoa, tea, and coffee. Those which contain very little are meat, milk, eggs, butter, cereals, rice, asparagus, cucumbers, mushrooms, lettuce, cauliflower, cabbage, pears, peaches, grapes, and melons.

The increased elimination of oxalic acid may or may not be associated with a deposition of crystals of calcium oxalate. An increased elimination is observed in cases associated with irregular activity of the gastro-intestinal tract. These cases usually show many types of nervous disorder, especially neurasthenia, and are characterized by the large deposit of oxalate crystals in the urine. To this condition has been given the name *oxaluria*, which can hardly be dignified as a definite pathologic entity. No inference can actually be drawn regarding the degree of elimination of oxalic acid from the appearance of a deposit in the urine, as it has been shown by Fürbringer that the urine may contain a large amount of oxalic acid without a sediment of calcium oxalate crystals being formed.¹ Such cases, however, should be watched more or less closely as separation of calcium oxalate may occur within the pelvis of the kidney and lead to the formation of a calculus. In cases of jaundice a marked oxaluria may be observed which is directly referable to the associated cholemia. In occasional cases of diabetes mellitus the elimination of oxalic acid may be much increased.

Quantitative Determination.

Baldwin's Method.²

Five hundred c.c. of a mixed 24-hour specimen of urine are treated with 150 c.c. of 95 per cent. alcohol and the mixture set aside for 48 hours to allow the calcium oxalate to precipitate. It is then filtered, each particle of the precipitate being transferred to the filter by means of hot water and a rubber-tipped glass rod. This precipitate is then washed with hot water and later with 1 per cent. acetic acid. The precipitate is then washed from the filter-paper by a stream of dilute hydrochloric acid from a wash-bottle until every trace of the precipitate is removed from the filter and dissolved. The filter is then washed with hot water until the washings are no longer acid in reaction. The hydrochloric acid solution and the washings are evaporated to about 20 c.c., a little calcium chlorid solution is added, the solution is neutralized with ammonia, is then rendered slightly acid with acetic acid, and 95 per cent. alcohol added in an amount equal to one-half the volume of the liquid. The mixture is then set aside for 48 hours, after which the precipitate of calcium oxalate is collected on an ash-free filter, washed with cold water and dilute acetic acid until free from chlorids, and the filter with its contents is incinerated in a weighed platinum crucible. This latter process is first carried out over a Bunsen burner and later over a blast-lamp. The crucible is then dried in a desiccator and weighed. The difference in weight represents

¹ Williams and Williams (Arch. Diagnosis, 1913, VI, 263), who show the relationship of obscure pain and hematuria to the excretion of small crystals of calcium oxalate. Fittipaldi, Rif. Med., 1915, XXX, 981; Sedgwick, Am. Jour. Dis. Child., 1915, X, 414; Hernando, Siglo Med., 1917, LXIV, 50; Fittipaldi, Rif. Med., 1917, XXXII, 1173.

² Jour. Exper. Med., 1903, V, 27. See, also, Bau, Biochem. Ztschr., 1921, CXIV, 221; Salkowski, Ibid., 1921, CXVIII, 259.

the amount of calcium oxid obtained from 500 c.c. of urine. Each gram of this oxid represents 1.6 grams of oxalic acid.

(4) Ferments.

Several ferments¹ have been demonstrated in the normal and pathologic urine, but do not seem to have any great clinical importance at the present time.

Pepsin.

This ferment is present in practically every specimen of urine. It has been found by Grober, Gehrig, Grützner, Mathes, Stadelmann, and others. It seems to be absent or very much diminished in cases of typhoid fever, gastric carcinoma, and hypoacidity.² In cases of pneumonia, Lenobel and Kun and Lochbihler have observed the presence of a ferment, which seemed to be pepsin in increased amounts. Scola reports a diminution of the normal pepsin content in severe diseases of the nervous system.

Diastase.

Traces of this ferment are found in normal urine. It is increased by a carbohydrate-rich diet and diminished by a carbohydrate-poor régime. Wohlge-muth³ believes an increase is associated with a disturbance of pancreatic function. It is lessened in nephritis. In diabetes the diastase varies with the sugar output.

Lipase.

This ferment is present normally only in minute traces. It is found, however, in cases of hemorrhagic pancreatitis, jaundice, and in diabetes mellitus. It may be detected by the method of Kastle and Loevenhart which is as follows: In each of three flasks are placed 5 c.c. of urine. One of the flasks is boiled to destroy the ferment which may be present. To a second flask are added a few drops of phenolphthalein solution and the acidity determined by titration with tenth-normal sodium hydrate solution. The amount of alkali necessary to neutralize the 5 c.c. of urine is then added to each of the other flasks. To these flasks are then added 0.25 c.c. of ethyl-butyrate and 0.1 c.c. of toluol, the flasks being then placed in the incubator at 37°C. for 24 hours. To each of these flasks there is then added $\frac{1}{2}$ c.c. more tenth-normal hydrochloric acid than the amount of tenth-normal alkali previously added.

¹ See Lindemann, *Ztschr. f. klin. Med.*, 1912, LXXV, 58; Kahn and Brim, *Am. Jour. Obs. and Dis. Women and Child.*, 1915, LXXI, 39, have studied the urinary catalase but find nothing of diagnostic value.

² See von Kozawa, *Internat. Beitr. z. Path. u. Ther., d. Ernährungsstör.*, 1912, IV, 44; Fuld and Hirayama, *Ztschr. f. exp. Path. u. Ther.*, 1912, X, 248; also, Tachau, *Ztschr. f. klin. Med.*, 1912, LXXVI, 167; Okada, *Mitt. a. d. Med. Fakultät, Tokio*, 1914, XI, 293; Fernandex-Arroyo, *Siglo Med.*, 1916, LXIII, 435.

³ *Biochem. Ztschr.*, 1909, XXI, 432; *Berl. klin. Wchnschr.*, 1910, XLVII, 92 and 1444. See, also, Corbett, *Jour. Obst. and Gynec. Brit. Emp.*, 1913, XXIII, 227; Neumann, *Deutsch. Arch. f. klin. Med.*, 1913, CXI, 164; and Geyelin, *Arch. Int. Med.*, 1914, XIII, 96; Mariño, *Semana Med.*, 1914, XXI, 907; Lister, *Brit. Med. Jour.*, 1914, II, 584; Brown and Smith, *Bull. Johns Hopkins Hosp.*, 1914, XXV, 213; King, *Am. Jour. Physiol.*, 1914, XXXV, 307; Kahn and Brim, *Am. Jour. Obs.*, 1915, LXXI, 39; Rowntree, Marshall and Baetjer, *Arch. Int. Med.*, 1915, XV, 543; Stocks, *Quart. Jour. Med.*, 1916, IX, 216; McClure and Pratt, *Arch. Int. Med.*, 1917, XIX, 568. Wallis, *Quart. Jour. Med.*, 1920, XIV, 57; *Brit. Med. Jour.*, 1920, II, 273.

The mixture is then shaken out with 50 c.c. of ether and 25 c.c. of alcohol to remove the butyric acid. This is then titrated with tenth-normal sodium hydrate, each c.c. of tenth-normal sodium hydrate representing 0.0088 gram of butyric acid.¹

(5) **Mucin-like Substances.**

(a) **Mucin.**

True mucin is present in traces in practically all urine. It is found both as an insoluble portion which forms the nubecula and as a soluble portion which is much smaller in amount than the insoluble form, is precipitated by acetic acid, but is easily soluble in a slight excess of the acid. This form of protein is derived from the urinary passages and has practically no pathologic importance, although it may be much increased in catarrhal conditions of the urinary tract in which it may appear as a gelatinous ropy material, or in rare cases in the form of casts of the ureter or urethra from 1 to 10 cm. long and 3 to 4 mm. thick. These cases are rare and have been reported by von Jaksch under the name of "ureteritis membranacea" and by Frank under the name of "pyelitis productiva."

Mucin is precipitated by the ordinary reagents for albumin (to be discussed later), but is soluble in an excess of these reagents, so that it need not be mistaken for albumin. It is frequently confused with the "nucleo-albumins," but may be distinguished, chemically, by the fact that it contains no phosphorus and gives on heating with acids a substance which reduces copper solutions.

(b) **Nucleo-albumin.**

The large majority of specimens of urine contain a substance (other than true mucin) which is precipitated on the addition of cold acetic acid. Dilute acetic acid does not dissolve the precipitate, so that true mucin is excluded, as the latter is usually dissolved even by dilutions of acetic acid which do not precipitate the other bodies. The reaction with acetic acid is somewhat intensified if the urine be diluted.

Practically every normal urine contains traces of such substances, which will give a precipitate with acetic acid, especially if the salts be removed by dialysis. In all probability this body, found in normal urines, is either euglobulin or a mixture of this protein with fibrinogen. It has been called "nucleo-albumin," but this is in all probability a misnomer. It is probably true that real nucleo-albumin is never a normal constituent of the urine. In this connection it must be stated that much confusion exists regarding the nature of true nucleo-albumin.² This substance has been considered to be identical with nucleo-protein, but with absolutely no basis of chemical facts. Nucleo-protein (true nuclein) is a combination of protein with the prosthetic group, nucleinic acid, which splits up into phosphoric acid and purin bodies; while nucleo-albumin (pseudo-nuclein) is a combination of protein with paranucleinic acid, which is rich in phosphorus, but does not yield purin bases on hydrolysis.

¹ See Quinan, *Jour. Med. Research*, 1915, XXXII, 45.

² See Jones, "Nucleic Acids," Longmans Green & Co., New York, 1914.

Mörner¹ believes that most of the so-called "nucleo-albumin" is a compound of true serum-albumin with an albumin-precipitating body formed on addition of acetic acid. He showed that there were three such precipitating bodies present in the urine; chondroitin-sulphuric acid² was practically always present, nucleinic acid occasionally present, and tauro-cholic acid, which may be normally present in traces, but in certain pathologic conditions is much increased. He believes that these precipitating bodies are normally present in excess and, therefore, any increase of a precipitate on addition of the acetic acid would mean an increased excretion of albumin. The more these precipitating bodies predominate the more the precipitate resembles "nucleo-albumin."

From the pathologic standpoint what has been called true "nucleo-albumin" appears in conditions destroying the integrity of the epithelium of the uriniferous tubules or of the bladder as well as in conditions associated with the excretion of pus in the urine. Thus we would expect to find such a body in acute nephritis, whether the result of bacterial or of exogenous toxins, in the acute febrile diseases, in renal hyperemia, in leukemia, in acute yellow atrophy, and in obstructive jaundice, in which case this body is derived doubtless from the bile. In cases of nephritis this body, precipitable by acetic acid in dilute solution, may precede and follow the true albuminuria. In amyloid kidney this body seems to be the chief type of protein present. In orthostatic albuminuria this substance may be the only protein present and may persist after the others have cleared up. In cases in which the urine contains a large number of epithelial cells, casts, and pus-cells, Matsumoto finds a substance precipitable by acetic acid, but only in very small amounts. This finding would seem to indicate that nucleo-albumin, if such ever occurs, is at least not an indication of cellular origin from increased epithelial desquamation. This body may be found in general catarrhal conditions of the urinary tract, as in cystitis or pyelitis, but in such cases we are more apt to obtain true mucin.

In the above discussion the writer has not attempted to differentiate these bodies, as the reports in the literature have little importance beyond the fact that a body precipitable by acetic acid was obtained. Each worker has named this body as he understood it and in many cases has had no definite basis for such conclusion. To remove it from the urine, add a solution of lead acetate and filter; the precipitated phosphates and chlorids carry down this protein.

(6) Pigments and Chromogens.

(a) Urochrome.

This pigment is the chief coloring matter of normal urine, imparting a yellow, orange, or a brownish color to the urine, depending upon its concentration. It is closely related to urobilin, especially the so-called normal urobilin of MacMunn, as this latter body may be readily converted into uro-

¹ Skand. Arch. f. Physiol., 1895, VI, 332.

² See Levene and La Forge, Jour. Biol. Chem., 1913, XV, 69 and 155; also, Pollitzer, Deutsch. Med. Wchnschr., 1912, XXXVIII, 1538; Med. Klin., 1913, IX, 2101; Levene and La Forge, Jour. Biol. Chem., 1914, XVIII, 123; Ibid., 1915, XX, 433; Levene and López-Suárez, Ibid., 1918, XXXVI, 105; Dietl, Wiener klin. Wchnschr., 1921, XXXIV, 133.

chrome by evaporation of its aqueous ethereal solution. Pelkan¹ believes that there is a causal relation between the output of urochrome and the degree of protein metabolism, while Roaf² regards urochrome as a derivative of chlorophyll. It is, in all probability, a mixture of one or more pigments, contains about 4 per cent, of nitrogen, and is free from iron. It is readily soluble in water and alcohol; sparingly soluble in acetic ether, amyl alcohol, and acetone; insoluble in ether, chloroform, and benzol.

An increase of urochromogen, the precursor of this pigment, seems to be of some clinical importance. Weisz³ has introduced the following test for its detection. Place the suspected urine in a test-tube and dilute with three times its volume of water. Mix well and add 3 drops of a 1 to 1000 aqueous solution of potassium permanganate. Shake well. A yellow tint or an increase in the yellow tone of the mixture indicates a positive reaction. This reaction is an indication of a toxic metabolic disturbance and is given in cases with high fever, especially in pulmonary tuberculosis and typhoid fever. It is never seen normally and, rarely, in carcinoma. A positive reaction is a contra-indication to the use of tuberculin in tubercular cases.

(b) Uroerythrin.

This pigment is a constituent of a large majority of normal urines, although it is probable that it indicates a slight deviation from the normal. It has been called rosacic acid by Prout and purpurin by Golding-Bird. To this pigment is due the salmon or brick-red color which the urinary sediments take in highly concentrated febrile urines. Normally, it may not be present in sufficient amount to color the urine, but under pathologic conditions it may impart a deep orange tint to this fluid. It is soluble in amyl alcohol, slightly soluble in acetic ether and absolute alcohol and very difficultly soluble in water.

This pigment appears to be increased on a meat diet, after severe exercise, profuse perspiration, or by irregular digestion. Pathologically, it is observed especially in cases of hepatic insufficiency, in chronic cardiac and pulmonary disease, in acute articular rheumatism, in malarial fever, and in general acute febrile diseases. In typhoid fever one does not find this pigment as frequently as in most other acute febrile conditions.

¹ Journ. Biol. Chem., 1920, XLIII, 237.

² Biochem. Jour., 1922, XV, 687.

³ Med. Klin., 1910, VI, 1661; Biochem. Ztschr., 1911, XXX, 333; Wien. klin. Wchnschr., 1912, XXV, 1183. See, also, Hefebower, Am. Jour. Med. Sc., 1912, CXLIII, 221; Paranhos and Gidlot, Brazil-med., 1913, XXVII, 101; Vitry, Rev. internat. de la tuberc., 1913, XXIII, 325; Skorzewski, Ztschr. f. exper. Path. u. Therap., 1913, XIV, 113; Salustri, Policlinico, 1913, XX, 1729; Nicola, Gazz. d. osp., 1913, XXXIV, 1465; Keim and Vigot, Presse méd., 1914, XXII, 153; Martelli and Pizzetti, Policlinico, 1914, XXI, 182; Gullbring, Hygiea, 1914, LXXVI, 590; Metzger and Watson, Jour. Am. Med. Assn., 1914, LXII, 1886; Schäfte, Ibid., 1914, LXIII, 1294; Pignacca, Gazz. d. osp., 1914, XXXV, 353; Tuliato, Ibid., 665; Balduzzi and Ballero, Ibid., 2053; Rhein, Münch. med. Wchnschr., 1914, LXI, 2355; Bruni, Gazz. d. osp., 1915, XXXVI, 401; Ferrannini, Riforma Med., 1915, XXXI, 477; Halbey, Med. Klin., 1915, XI, 833; Mühlens, Münch. med. Wchnschr., 1915, LXII, 1067; Pulay, Ibid., 1009; Genoese, Policlinico, 1915, XXII, 558; Biesenthal, Ill. Med. Jour., 1915, XXVIII, 344; Pulay, Münch. Med. Wchnschr., 1915, LXII, 1009; Svestka, Wien. klin. Wchnschr., 1915, XXVIII, 1054; Burgess, Jour. A. M. A., 1916, LXVI, 82; Cowen, Ibid., 791; Belgrano, Policlinico, 1916, XXIII, 276; Schwensen, Ugeskrift f. Laeger, 1917, LXXIX, 231; Lollini, Policlinico, 1917, XXIV, 308; Klare, Münch. med. Wchnschr., 1920, LXVII, 635; Weisz, Biochem. Ztschr., 1920, CII, 228; Ibid., 1920, CXII, 61; Wiener Arch. inn. Med., 1920, I, 358; Bonnert, Tubercle, 1921, II, 537; Haug, Deutsch. med. Wchnschr., 1921, XLVII, 1589.

(c) **Urobilin.**

This substance appears in the urine not as a free pigment, but in the form of the chromogen urobilinogen, which is decomposed into urobilin through the influence of the light. It is claimed that various types of urobilin are found, as for instance the normal urobilin of MacMunn and the pathologic urobilin of Jaffé. Whether these are really different bodies is at present an unsettled question. Urobilin appears to be identical with the stercobilin of the feces and is not the same as the normal fecal hydrobilirubin. Much discussion has centered around the origin of this pigment. Passing through the stages of the hepatogenous, hematogenous, nephrogenous and histogenetic urobilinuria, the general consensus of opinion seems at present to be that most of the urinary urobilin is of enterogenous origin.¹ According to this theory, the pathogenesis of urobilinuria may be presented as follows:

"The liver-cell, both in normal and abnormal conditions, forms only bilirubin from the blood pigment. Providing there is no marked obstruction to the passage of bile into the intestine, the bilirubin is acted upon by bacteria which reduce it so completely to urobilin that only traces of bilirubin appear in the feces. A part of the urobilin is absorbed and is excreted in the urine, while traces appear in the bile and in pathologic transudates. When bacterial action is excluded, as in the new-born, no urobilin is found in the urine. Further, when bile is not present in the intestine, as in cases of absolute occlusion of the ductus choledochus, urobilinuria does not occur. It is sparingly excreted when the production of biliary pigment is diminished, as in hunger, while the amount is small or at most normal in cases of incomplete exclusion of bile from the intestine. On the other hand, the amount excreted may reach abnormal limits if a preceding obstruction, accompanied by stasis, has been overcome, and bile flows freely into the intestine. Likewise the quantity may be abnormally large if the production of biliary pigment from the red blood-corpuscles increases as a result of infection and intoxication, or of hepatic lesions, such as cirrhosis and cyanotic induration. In these cases the bile is tenacious and the condition may give rise to jaundice although it is seldom that the stasis is so great that the bile is completely shut off from the intestine. Indeed, in most cases, owing to the excretion of excessive pigments in the bile (pleiochromia), a more than normal amount of pigment passes into the intestine, and as a result of this there arises a marked urobilinuria with a mild degree of biliary stasis. In many cases the stagnation of bile is great enough to cause a passage of biliary pigment from the blood into the urine, leading to a marked urobilinuria, a mild degree of bilirubinuria, and a yellow-tinting of the tissues. In other cases the absorption of bile is so slight that only yellowing

¹ See Wilbur and Addis, *Jour. Am. Med. Assn.*, 1912, LIX, 929; also, Fromholdt and Nersessoff, *Ztschr. f. exper. Path. u. Therap.*, 1912, XI, 400; Wilbur and Addis, *Arch. Int. Med.*, 1914, XIII, 235; Feldner, *Centralbl. f. d. Grenzgeb. d. Med. u. Chir.*, 1915, XIX, 163; Peters, *Ned. Tijdschr. v. Geneesk.*, 1916, I, 1602; Brulé, *Presse Méd.*, 1919, XXVII, 714; *Jour. pharm. chim.*, 1920, XXII, 401; Strauss and Hahn, *Zentralbl. f. inn. Med.*, 1920, XLI, 193; Labbé and Carrié, *Presse Méd.*, 1920, XXVIII, 353; Brandt, *Ugesk. f. Laeger*, 1920, LXXXII, 1083; Whipple, Hooper and Robschett, *Am. Jour. Physiol.*, 1920, LIII, 167; Bauman, *Arch. Int. Med.*, 1921, XXVIII, 475; Brulé and Garban, *Presse Méd.*, 1921, XXIX, 533; *Rev. de Méd.*, XXXVIII, 583.

of the tissues results, yet the concentration of pigment in the blood does not suffice to permit of its excretion by the kidneys, so that marked urobilinuria and yellowing of the tissues without bilirubinuria ensues" (Weintraud).

Urobilin is found in febrile conditions, chronic passive congestion, lead-poisoning, cases in which extravasation of blood into the tissues occurs, in any condition associated with marked hemolysis, hepatic cirrhosis, and in the cases of jaundice outlined above by Weintraud. It has been noticed in increased amounts in Addison's disease, extrauterine pregnancy, hemophilia, and in secondary syphilis.¹

The presence of an increased amount of urobilin usually causes a dark-yellow color of the urine, the foam in such cases being colored, more perhaps due to the presence of other pigments than to urobilin itself.

Urobilin is soluble in ethyl alcohol, amyl alcohol, and chloroform, and slightly soluble in ether, acetic ether, and in water. If an acid solution of urobilin be examined with a spectroscope, it shows a broad absorption band to the right of *E*, the left-border of which reaches nearly to *b*, while the right border encloses *F*. If the solution be alkaline, the spectrum shows a less broad absorption band between *E* and *F* enclosing *b*. This solution is best made as follows: Ten to 20 c.c. of the urine are acidulated with a few drops of hydrochloric acid and shaken out with from 6 to 10 c.c. of amyl alcohol, which then shows the characteristic spectrum of acid urobilin. If to a small portion of this amyl alcohol solution be added a few drops of 1 per cent. solution of zinc chlorid, which has been strongly alkalinized with ammonia, a beautiful green fluorescence appears.² With Ehrlich's dimethyl-amido-benzaldehyde reaction (p. 371) this pigment gives a distinct red color.³

(d) Indican.⁴

In the decomposition of protein occurring in the intestinal canal indol (C_8H_7N) and skatol (C_9H_9N) are found among the products of this bacterial cleavage. These substances are absorbed and oxidized in the blood to indoxyl (C_8H_7NO) and skatoxyl (C_9H_9NO). These bodies are then conjugated with sulphuric acid forming indoxyl and skatoxyl sulphuric acids, after which they are excreted in the form of the potassium salt, indoxyl potassium sulphate ($C_8H_6NO-SO_2-OK$) and skatoxyl potassium sulphate ($C_9H_8NO-SO_2-OK$). To the former of these is given the name indican. This chromo-

¹ See Simon, *Med. Klin.*, 1913, IX, 1164; Hildebrandt, *Arch. f. Verdauungskr.*, 1913, XIX, 442; Kamsarakau, *Med. Obozr.*, 1913, LXXIX, 559; Molnar, *Virchow's Arch. f. path. Anat.*, 1913, CCXIII, 433; Hausmann, *Ztschr. f. exper. Path. u. Therap.*, 1913, XIII, 373; Litzenberg, *Am. Jour. Obs. and Dis. Women and Child.*, 1916, LXXIII, 228; Kirch, *Wien. klin. Wchnschr.*, 1916, XXIX, 1238; Barnard, *Jour.-Lancet*, 1917, XXXVII, 80; Boyd, *Jour. Lab. and Clin. Med.*, 1919, IV, 495; Hausmann and Howard, *Jour. A. M. A.*, 1919, LXXIII, 1262; Reynolds, *Semana Méd.*, 1919, XXVI, 735; Hansen, *Ugesk. f. Laeger*, 1920, LXXXII, 415; Dietl and Szigeti, *Med. Klin.*, 1920, XVI, 444; Mordre, *Norsk Mag. f. Laegevidensk.*, 1921, LXXXII, 202.

² See Edelman, *Wien. klin. Wchnschr.*, 1915, XXVIII, 978; Marcussen and Hansen, *Jour. Biol. Chem.*, 1918, XXXVI, 381; Pittarelli, *Rif. Med.*, 1921, XXXVII, 492; Hausmann, *Münch. med. Wchnschr.*, 1921, LXVIII, 1558; Adler, *Deutsch. Arch. f. klin. Med.*, 1922, CXXXVIII, 309.

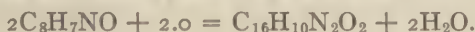
³ See Hari, *Biochem. Ztschr.*, 1921, CXVII, 41.

⁴ See Baar, *Die Indicanurie*, Wien, 1912; *New York Med. Jour.*, 1914, XCIX, 669.

gen is present in much larger amounts than is the skatoxyl compound so that our discussion will include principally the former.

The absolute amount of indican occurring in the urine depends upon the amount of decomposition occurring in the intestine. The largest amounts are naturally observed in health following a meat diet; while the urine may be practically free from indican on a vegetable or a milk diet. The pathologic variations may be summed up as follows. An increased elimination of indican is observed in all diseases which are associated with an increased intestinal decomposition.¹ This decomposition usually occurs in the large intestine, but may at times take place in the small bowel, in which cases the degree of indicanuria may be greater owing to the increased absorptive power of the small bowel. Although many writers state that indicanuria is not seen in cases of simple constipation, the writer must take exceptions to these statements as some of his most intense and persistent cases of indicanuria have been found in uncomplicated constipation. Secondly, an increased excretion of indican is observed in cases showing diminished peristalsis, as, for instance, in ileus and peritonitis. These cases frequently show an intense reaction.² If the obstruction leading to ileus be in the large bowel, indican is either absent or appears much later than is the case if the small bowel be involved. Lastly, in any condition associated with protein decomposition anywhere in the system, as, for instance, in empyema, putrid bronchitis, abscess formation, etc., indican may be much increased.

The color of the urine is usually normal when voided, although a large amount of indican may be present. In some cases oxidation of this chromogen has occurred within the system and the urine appears greenish or even blue when voided. If the urine be allowed to stand until decomposition occurs, a reddish or bluish metallic-like scum may be observed, due to the conversion of the indican into indigo-blue. Most of the tests for the presence of indican in the urine depend upon the oxidation of the indican, according to the following equation (this body having been previously decomposed by concentrated HCl into indoxyl and sulphuric acid).



Tests for Indican.

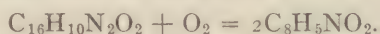
Jaffé's Test.

A few c.c. of urine are treated with an equal volume of concentrated hydrochloric acid, two or three drops of a strong solution of calcium hypo-

¹ See Morgan, *Am. Jour. Med. Sc.*, 1912, CXLIV, 827; also, Benedict, *Arch. Diagnosis*, 1912, V, 150; Sherwin and Hawk, *Biochem. Bull.*, 1914, III, 416; *Jour. Am. Chem. Soc.*, 1914, XXXVI, 1779; Hoppe-Seyler, *Deutsche Med. Wchnschr.*, 1916, XLII, 1213; Jolles, *Med. Klin.*, 1919, XV, 814; Underhill and Simpson, *Jour. Biol. Chem.*, 1920, XLIV, 69; Dea-man, *Southern Med. Jour.*, 1920, XIII, 232; Dohi, *Japan Med. World*, 1920, X; Wilson, *Jour. Lab. and Clin. Med.*, 1920, V, 515; Becher, *Deutsch. med. Wchnschr.*, 1921, XLVII, 42; Bonar, *Am. Jour. Dis. Child.*, 1921, XXI, 406; Nigro, *Riv. di Clin. Pediat.*, 1921, XIX, 278; Cantelli, *Rif. Med.*, 1921, XXXVII, 993.

² Although there appears to be no definite relation of indicanuria and albuminuria, the writer has rarely failed to observe large numbers of casts in urines showing marked indican reactions. Whipple, Rodenbaugh and Kilgore (*Jour. Exper. Med.*, 1916, XXIII, 123), Whipple, (*Jour. A. M. A.*, 1916, LXVII, 15) and Whipple and Cooke (*Jour. Exper. Med.*, 1917, XXV, 461 and 479) have shown that the intoxication in intestinal obstruction is due to a primary proteose. See, however, Dragstedt, Moorhead, and Bursky, *Proc. Soc. Exper. Biol. and Med.*, 1916, XIV, 17.

chlorite (bleaching powder or "chlorid of lime") are added and the contents mixed. Two c.c. of chloroform are then added and the tube inverted several times. In this process the indican is oxidized to indigo-blue which is taken up by the chloroform. The depth of the blue coloration of the chloroform will serve as an approximate estimate of the amount of indican present. In absolutely normal urine no blue coloration or, at most, a faint bluish tinge is observed. Care must be taken in this test to avoid an excess of the hypochlorite, as this will convert the indigo-blue into isatin which is distinctly yellow, according to the following equation:



Obermayer's Test.

A few c.c. of urine¹ are mixed with an equal volume of Obermayer's reagent (a 0.2 per cent. solution of ferric chlorid in concentrated hydrochloric acid) and the solutions mixed by repeatedly inverting the tube. A few c.c. of chloroform are then added and the tube inverted as before. The presence of any appreciable amount of indican is indicated by a dark brown to black coloration of the urine and the later absorption of this color by the chloroform, which becomes a more or less deep shade of blue. If the urine be very dark in color or if bile pigment be present, the pigments may be removed by adding a solution of lead subacetate and filtering. If potassium iodid be present this test yields a red color which will disappear on addition of sodium thiosulphate solution. Salicyluric acid gives a violet coloration. If formalin be used as a preservative, indican is incapable of detection by any of the tests given. Thymol-containing urines give a violet coloration to the chloroform extract. This color is destroyed by sodium hydroxide or thiosulphate.² Jolles³ has made this fact the basis of a new test for indican in the urine.

Instead of using the Obermayer reagent as such, the urine may be mixed with the hydrochloric acid and one or two drops of a 10 per cent. solution of ferric chlorid may then be added to the mixture. This test is a much more reliable one than is the Jaffé test, as the latter is very prone to carry the oxidation to the isatin stage rather than to the indigo-blue phase.⁴

Skatoxyl-potassium Sulphate.

A certain amount of skatoxyl-potassium sulphate is formed along with the indican. In some cases for reasons not well understood, this pigment or one closely allied to it appears in the urine in excess of the indican, so that the above test may give distinct red colorations of the chloroform instead of the usual blue.⁵ Just exactly what pigment causes these red variations is doubtful. Some of this pigment is always present along with the indican

¹ Askenstedt, Jour. Lab. and Clin. Med., 1917, II, 578, advises warming the urine before addition of the reagent.

² Rosenbloom, New York Med. Jour., 1913, XCVIII, 814.

³ Ztschr. f. physiol. Chem., 1913, LXXXVII, 310. See, also, Stanford, Ibid., 188 and Ibid., 1913, LXXXVIII, 47; also, Edes, Arch. Diagnosis, 1913, VI, 152; Rossi, Policlinico, 1914, XXI, 297; Cantelli, Gazz. d. osp., 1915, XXXVI, 833; Jolles, Ztschr. f. physiol. Chem., 1915, XCIV, 79; Ibid., 1915, XCV, 29.

⁴ See Natonek, Zentralbl. f. inn. Med., 1913, XXXIV, 1124.

⁵ See de Jager, Nederl. Tijdschr. v. Geneesk., 1910, II, 1873; Sieber, Casopsis, lek. cesk., 1922, LXI, 6 (Abs. Jour. Am. Med. Assoc., 1922, LXXVIII, 932).

and may be extracted either with hot water or with a mixture of alcohol, ether and water. To this red pigment has been given the name indigo-red, skatoxyl-red, uro-rubin, uro-rhodin, and several others.

If the urine be heated on applying Jaffé's test, a dark red coloration is observed, while if Obermayer's test be used the coloration will be of a reddish-violet.

Rosenbach's Test.

A few c.c. of urine are boiled and concentrated nitric acid added drop by drop during the boiling. The urine takes a deep red color and the foam appears bluish-red. If the nitric acid be much in excess the urine will assume a yellowish-red color and the foam a distinctly yellow tint. If sodium hydrate or ammonia be now added drop by drop, a bluish-red precipitate is observed which is soluble in an excess of the alkali with a brownish-red coloration. This test is due to the presence of indigo-red. Its clinical significance is the same as that of indican. This pigment may be obtained directly from the urine by neutralizing it with sodium hydrate and shaking out with ether, when the ether will take a distinct red color.

Quantitative Determination.

Wang's Method.

The principle of this method is the decomposition of indican and its oxidation to indigo-blue. This compound is then transformed into indigo-sulphuric acid, which is directly determined by titration with potassium permanganate solution.

Technic.

A preliminary determination of the relative amount of indican is made with Obermayer's reagent. If a strong reaction is obtained, from 25 to 100 c.c. of urine are used, while if the reaction be slight 200 to 500 c.c. are necessary. The urine should be acidified with acetic acid, unless its reaction be already acid.

Fifty c.c. of urine, or a larger amount if the conditions mentioned above obtain, are treated with 5 c.c. of a 20 per cent. solution of lead acetate, or one-tenth the volume of urine in case a larger amount of urine be taken. The urine is then filtered and a large and accurately measured portion of the filtrate is treated, in a separatory funnel, with an equal volume of Obermayer's reagent. This mixture is then shaken out with chloroform, using 30 c.c. of this menstruum and shaking for one minute. At least four such chloroform extractions should be made, more if the chloroform still extracts indigo-blue from the mixture. The chloroform extract is placed in a small flask and the chloroform distilled. The residue in the flask is dried for a few minutes on the water-bath to remove the last traces of the chloroform, and is washed either with hot water or with a mixture of equal parts of alcohol, ether, and water. These solvents remove the red coloring matter, leaving the indigo-blue undissolved. The extract is filtered through a small filter and the indigo-blue completely transferred to the filter, after which it is thoroughly washed with hot water. This indigo-blue is dissolved on the filter with boiling chloroform, the filtrate being

allowed to run into the original flask. The chloroform is again distilled and the residue dried on the water-bath. This purified indigo-blue is dissolved in 10 c.c. of concentrated sulphuric acid and the solution diluted to 100 c.c. with water.

This solution of indigo-sulphuric acid is then titrated with standard potassium permanganate solution of such a strength that 1 c.c. will represent approximately 0.0062 gram of indigo-blue. This solution of potassium permanganate contains about 3 grams of potassium permanganate to the liter. Its titer is determined before use by titrating against a solution of pure indigo-blue in sulphuric acid. In making this titration the concentrated solution is not used, but a dilute one made by diluting 5 c.c. of the stronger permanganate solution to 200 c.c. (each c.c. of which will represent 0.000155 gram of indigo-blue). In this titration the blue color of the indigo-sulphuric acid does not change to any extent on the addition of the first drops of the permanganate solution, but gradually turns greenish and then becomes yellowish or entirely colorless. The amount of indigo-blue in the urine used is readily ascertained by multiplying the number of c.c. of permanganate solution used by the amount of indigo-blue represented by the titer of the solution (in the writer's laboratory 1 c.c. of the diluted solution equals 0.000155 gram of indigo-blue).

It has been found by Ellinger that only about 87 per cent. of the theoretical yield is obtained by this method, owing, probably, to the simultaneous formation of isatin from the indigo-blue. The average daily excretion of indican, as revealed by this test, ranges between 5 and 20 mg.

Folin's Method.

This method has not, as yet, been extended so as to give absolutely quantitative results. Its principle is the comparison of the color given when urine is treated with Obermayer's reagent with that of Fehling's solution as a standard. To this standard has been given the arbitrary value of 100.

Exactly one one-hundredth of the 24-hour specimen of urine is taken for each determination and treated with an equal volume of Obermayer's reagent. The indigo-blue is then extracted with 5 c.c. of chloroform until all of the pigment has been dissolved. With the chloroform solutions are then made colorimetric comparisons with Fehling's solution, using either the Duboscq or Sargent instrument. Folin finds the average indican excretion, on this basis, from a diet of 119 grams of protein to be 77, while on a nitrogen-free diet no indican is found.

(e) Uroroseinogen.

This chromogen, shown by Herter¹ to be indol-acetic acid, is converted by oxidation into the pigment urorosein, which is soluble in ethyl and amyl alcohol but insoluble in chloroform, ether and benzol. If the urine stands for some time bacterial action produces this pigment, although an alkaline reaction may destroy the coloration. Its amyl-alcoholic solution shows a sharp narrow absorption band between D and E.² Although this pigment appears

¹ Jour. Biol. Chem., 1908, IV, 107, 239 and 253.

² See Riesser, Inaug. Dissert., Königsberg, 1911.

in normal urine only in traces, it may be markedly increased by a strict vegetable diet. Pathologically, it appears in cases of severe gastric and intestinal disturbances, tuberculosis, pernicious anemia, nephritis, severe chlorosis, diabetes mellitus, carcinoma, osteomalacia, typhoid fever and dementia praecox.¹ It has, therefore, little differential value although it is, often, as significant as indican of excessive protein decomposition in the bowel.

Test for Urorosein (indol-acetic acid): If 5 c.c. of urine be mixed with 5 c.c. of chemically pure HCl and a few drops of a 1 per cent. solution of sodium nitrite a characteristic rose-red coloration develops, which is due to oxidizing rather than nitrifying action. This pigment also gives a red coloration with Ehrlich's dimethyl-amidobenzaldehyd reaction.

(B) Abnormal Composition.

(1) Proteins.

There has been much discussion as to whether a true physiologic proteinuria occurs.² Without going into a discussion of the subject the writer wishes to state his belief that a true physiologic proteinuria occurs, but that such may never be detected by the usual clinical methods of examination. For our purposes, therefore, the presence of protein of one type or another in amounts amenable to detection by clinical methods must be considered pathological.

(a) Serum Albumin.

From the pathologic standpoint, serum albumin is the most important protein found in the urine. The amount excreted in 24 hours is variable and does not necessarily have any relation to the severity of the kidney lesion should one really exist. From 5 to 10 grams of this protein *per diem* may be regarded as a moderate pathologic excretion, while a lesser would be of little significance and a greater would be regarded as excessive. As large amounts as 40 grams have been observed, but such findings are exceptional. Mörner considers an excretion of albumin varying from 25 to 75 mg. per liter as a normal output, which amount would, however, escape the usual clinical tests.

As some of the more delicate tests for albumin, as for instance Spiegler's reagent, will show traces of albumin in practically every specimen of urine examined, we should limit our conception of albuminuria to those cases which react with the ordinary tests rather than with the most delicate. According to Hofmeister, the standard upon which one bases a judgment as to the presence of a pathologic albuminuria is the formation of a distinct albumin ring within three minutes after the urine and nitric acid are in contact in Heller's test (see below). Moreover, the term albuminuria should be limited to those cases in which there is some disturbance of the renal epithelium, especially of the glomeruli. This does not exclude those cases of purely functional albuminuria in which no distinct lesion of the kidney exists, as these cases are all associated with some abnormality of the excreting organ.

It not infrequently happens that albumin, as found in the urine, is derived

¹ See Ross, Arch. Int. Med., 1913, XII, 112 and 231.

² See Backman, C. R., soc. biol., Paris, 1916, LXXIX, 339.

from some portion of the urinary tract below the kidney, as when inflammatory exudates, blood, lymph, spermatic or prostatic fluids, pus and other extraneous material are mixed with the urine after it is excreted by the kidney.¹ To such cases is given the name *false* or *accidental albuminuria*, in contradistinction to the true albuminuria in which the albumin is present when excreted by this organ. Before reporting a finding as one of true albuminuria, it is absolutely imperative that extraneous albumin be excluded.

Functional Albuminuria.

Not infrequently do we find in perfectly healthy individuals a urine which is normal in every way with the exception of the presence of albumin, which is easily detected by our ordinary methods. This type of albuminuria is observed following severe muscular exercise beyond the point to which the subject is accustomed. Thus, in raw recruits under the severe forced marches during the early days of their service we find this type very prevalent. Leube states that 59 per cent. of such soldiers show a temporary albuminuria, which disappears after the subject becomes accustomed to the increased exercise.² It is a common occurrence to find such a functional albuminuria in football players, bicycle riders, crew men, and general athletes after every period of increased exertion. This question may be summed up by the statement that the excretion of albumin is simply dependent, under these conditions, upon the limit of endurance of the subject, practically everyone being able to produce an albuminuria if he overdoes in any way. Moreover, cold baths, excessive mental labor, and severe emotion may lead to quite an extensive albuminuria, especially in an individual with somewhat lessened resistance. According to Rem-Picci albuminuria is a constant finding after cold baths, different subjects reacting differently to the same stimuli. This albuminuria never lasts over 24 hours and may be associated with the appearance of casts and blood, as may also the type following increased exercise. The colder the bath and the longer the immersion, the more rapid the appearance of albumin.

Another type of functional albuminuria is that following the intake of a heavy protein meal. This is known as "*alimentary albuminuria*." It has been supposed to be due to filtration from the blood, the foreign protein being absorbed and excreted without intervening hydrolytic cleavage. Uhlenhuth,³ Inouye,⁴ and Croftan,⁵ working with the precipitin test, have found egg albumin in the blood and urine in these cases. Ascoli⁶ later demonstrated both serum and egg albumin by this test. Wilson⁷ using the fixation

¹ See Belfield (Jour. Am. Med. Assn., 1915, LXIV, 2040) for a discussion of vesicular albuminuria due to presence of both globulin and albumin derived from the seminal vesicles.

² See von Hecker, Berl. klin. Wchnschr., 1913, L, 1848; Bugge, Norsk Mag. f. Lægevidensk., 1913, LXXIV, 1601; Reber and Lauener, Corr.-Bl. f. Schweiz. Aerzte, 1915, XLV, 949; Mossy and Richet, Paris Medical, 1917, VII, 47; Bornstein and Lippmann, Ztschr. f. klin. Med., 1918, LXXVI, 344; MacLean, Jour. Royal Army Med. Corps, 1918, XXXI, 245 and 310; Parmenter (Boston Med. & Surg. Jour., 1920, CLXXXIII, 677) states that 8 per cent. of the students examined at Harvard showed albuminuria.

³ Deutsch. med. Wchnschr., 1900, XXVI, 734.

⁴ Deutsch. Arch. f. klin. Med., 1903, LXXV, 378.

⁵ New York Med. Jour., 1909, LXXXIX, 474.

⁶ Münch. med. Wchnschr., 1903, L, 201 and 1761; Ztschr. f. physiol. Chem., 1903, XXIX, 283.

⁷ Jour. Path. and Bacteriol., 1909, XIII, 484.

of complement test, found the main element to be of human origin. Recently Wells,¹ applying the anaphylaxis reaction,² has shown that sensitized animals do not react to egg albumin but show marked reactions to human protein. Owing to this disagreement of the anaphylaxis and precipitin tests, it would seem probable that the excreted albumin is derived from the blood rather than directly from the food, little if any unaltered food protein ever reaching the urine.³

The albuminuria observed in the new-born for the first few days of life, aside from the influence of feeding upon foreign proteins, is probably a further example of a true functional albuminuria. Likewise, the albuminuria observed in pregnancy is usually distinctly functional. About 50 per cent. of pregnant women show this albuminuria, little difference being observed between primiparæ and multiparæ. The kidney undoubtedly shows some functional disturbance in its attempt to eliminate the toxic products absorbed from the fetus.⁴

According to Senator⁵ such albuminurias as outlined above should be considered functional when slight in degree, transitory in character, occurring after unusual strain, either physical or mental, the subjects showing a further negative history after the removal of the direct stimulus. Whether this type is to be considered physiologic rests entirely upon the conception of the normal or abnormal nature of the stimuli leading to the excretion of albumin. It would seem to the writer that the term "physiologic albuminuria" would better be given up or at least used with great caution, as it is difficult to believe that the severe strain put upon the system and kidneys, in particular, can be distinctly physiologic.⁶ He is inclined to reserve this term for the excretion of the minimal amounts of albumin, which cannot be detected by the simpler clinical tests, and to regard the types of albuminuria, as discussed above, as "*functional*" (Pavy), or "*constitutional*" (Martius).

A second group of cases showing albuminuria is observed in which the excretion of albumin may persist for a period varying from days to months, may disappear for a variable space and then return for another indefinite length of time. To this type is given the name "*intermittent albuminuria.*" This is purely functional, no lesion of the kidney being manifest. The cases usually show a history of an acute infection or of an antecedent nephritis as the result of such infection. The most frequent cause, however, is an uncompensated heart lesion which may or may not be associated with a direct renal

¹ Jour. Am. Med. Assn., 1909, LIII, 863.

² See Schittenhelm and Weichardt, Ztschr. f. exper. Path. u. Therap., 1912, XI, 69; Pearce, Jour. Exper. Med., 1912, XVI, 349; also, Schloss, Am. Jour. Dis. Child., 1912, III, 341.

³ See Van Alstyne and Grant, Jour. Med. Research, 1911, XXV, 399; also, Van Alstyne, Arch. Int. Med., 1913, XII, 372; Salus, Biochem. Ztschr., 1914, LX, 1; Cameron and Wells, Arch. Int. Med., 1915, XV, 746; Bronfenbrenner, Andrews and Scott, Jour. Am. Med. 1915, LXIV, 1306; Longcope, Am. Jour. Med. Sc., 1916, CLII, 625; Hoobler, Am. Jour. Dis. Child., 1916, XII, 129.

⁴ See Bondi, J. and S., Arch. f. Gynäk., 1914, CII, 89; Macleod, Jour. Lab. and Clin. Med., 1917, II, 520.

⁵ Erkrankungen der Nieren, Wien, 1902.

⁶ See Wolf, Arch. Diagnosis, 1912, V, 260.

lesion. Not infrequently we find an hereditary intermittent albuminuria in those with a distinct neurotic family history.

The functional albuminuria may, at times, follow a definite course, disappearing and reappearing with such regularity that it has been styled "*cyclic albuminuria*." In this form the albumin usually disappears from the urine at night and when the patient is flat on his back, but reappears during the day or when the subject is erect. The terms "*orthostatic, orthotic, or postural albuminuria*" would, therefore, seem to be more appropriate than the former appellation. This type is dependent, according to Erlanger and Hooker, upon a lowering of the pulse pressure which constantly occurs when the individual changes from the recumbent to the erect position. Jehle and, more recently, Nothmann believe that this type is due to a lordosis, and style it, therefore, *lordotic albuminuria*.¹ Sonne and, recently, Rieser and Rieser have shown that this type of albuminuria is apparently due to renal stasis caused by compression of the left renal vein. Certain pathologic cases, as beginning nephritis, may show a cyclic albuminuria which may extend well into the period of recovery. In true cyclic cases the negative physical findings would lead us to class the condition among the functional albuminurias. If a case of this type becomes persistent and casts are frequently found, subsequent cardiovascular changes will usually appear, changing the case into one of true nephritis. The albumin in such cases usually appears in the urine after rising and reaches a maximum from noon to 3 or 4 o'clock, then gradually declines, disappearing from 8 to 10 p. m. If the subject changes his habits of life, the cycle of albuminuria will also change. Along with the variations in the excretion of albumin, the other urinary constituents fluctuate in the same manner, the sequence being, according to Teissier, increase in pigments, albumin, uric acid, and urea. A peculiarity of this type of albuminuria is that it may be even diminished by exercise and is less, therefore, after a hard day's work, differing from the albuminuria in nephritis or in the cardiac types.

While there has been much discussion as to whether such cases are not truly pathological, most of them at any rate must have a certain insufficiency of the renal epithelium. This may be due to changes in the circulation following change in posture, but it is rather surprising that increased exercise would not work more strongly in this way. Krehl regards these conditions as rela-

¹ See Hamburger, Wien. klin. Wchnschr., 1912, XXV, 262; also Fränkel (Deutsch. med. Wchnschr., 1912, XXXVIII, 7085), who finds the urinary acidity increased in these cases and states that, possibly, the albuminuria is due to abnormal production of acid as Fischer's theory assumes. See, also, Gomolitsky, Ztschr. f. klin. Med., 1913, LXXVII, 96; Reyher (Monatsschr. f. Kinderhkde., 1913, XII, 82), Dietl (Wien. klin. Wchnschr., 1913, XXVI, 258) and von Jagie (Ibid., 1356) believe that tuberculosis is the important factor in this condition. See, also, Wendenburg, Arch. f. Kinderhkde., 1913, LXII, 34; Jellie, Die Albuminurie, Berlin, 1914; Reyher, Pediatra, 1914, VI, 1; Steensma, Nederl. Tijdschr. v. Geneesk., 1914, LVIII, 248; Groenendijk, Ibid., 885; Barlocco, Gazz. d. osp., 1914, XXXV, 2021; Fischl and Popper, Jahrb. f. Kinderhkde., 1915, LXXXI; Zondek, Ztschr. f. klin. Med., 1915, LXXXII, 78; Jeanneret, Arch. de Méd. des. Ent., 1915, XVIII, 401; Holst, Norsk. Mag. f. Lægevid., 1915, LXXVI, 1377; Mason and Erickson, Am. Jour. Med. Sc., 1918, CLVI, 643; MacKay, Can. Med. Assoc. Jour., 1919, IX, 973; Apert and Vallery, Radot, Bull. de la soc. méd. des Hôp. de Paris, 1920, XLIV, 937; Saito, Japan Med. World, 1920, X; Sonne, Ztschr. f. klin. Med., 1920, XC, 1; Saito, Am. Jour. Dis. Child., 1921, XXII, 388; Harrison, Lancet, 1921, II, 991; Gram, Ugesk. f. Læger, 1921, LXXXIII, 1797; Rieser and Rieser, Jour. Am. Med. Assoc., 1922, LXXVIII, 644.

tively harmless as they do not usually show any subsequent history of nephritis; Broadbent does not believe such cases ever develop actual renal disease; while Senator insists that most of them are cases of nephritis. The patients showing this type of albuminuria are weak anemic individuals about the age of puberty, subject to fainting spells, with a heart showing intermittent attacks of dilatation and palpitation, and with a probable congenital weakness of the kidney (37.5 per cent. having a movable kidney). The adults are neurasthenics with distinct vasomotor paralysis.

A type of albuminuria has been observed in some patients with enlarged spleen, in which albumin is present when the patient is flat on his back but is absent when he is erect. This has been called "*hypostatic albuminuria*." Its pathogenesis is uncertain, but it can have only an indirect relation to the enlarged spleen, as many cases of splenic tumor do not show this albuminuria.

A still further type of functional albuminuria is known as "*albuminuria of adolescence*." This occurs between the ages of 14 and 16, and then disappears. It is different from the cyclic type, although this latter occurs in young people. The children are usually anemic, have a neurotic family history, have an unstable vasomotor system and, possibly, a congenital weakness of the kidney. In this class should be included the albuminuria shown by masturbating children or after sexual excess at this period. The kidney does not keep pace with the physical growth and activity of the system, so that the association with the unstable vasomotor system may account for the albuminuria (Emerson). According to Sutherland, movable kidney may be accountable for some of these cases, as he finds it present in one-third of his cases.

Febrile Albuminuria.

During the course of any acute fever, an albuminuria may be observed which is not associated with distinct changes in the renal parenchyma and disappears with the fall of temperature. The amount of albumin excreted may be small or great, depending upon the severity of the toxic action of the bacterial products.¹ In ordinary cases there is practically no inflammatory condition present in the kidney, the albuminuria being due to an ischemia and a later hyperemia. In some of the infectious fevers the influence of the toxins is so great that a true nephritis originates, as especially noted in scarlet fever and diphtheria. Any febrile albuminuria may pass into a true nephritis, so that the case must be closely watched for the appearance of symptoms indicating such a complication. In some cases, an increase in the albuminuria is observed during convalescence, when only traces were previously noted. This is known as "*colliquative albuminuria*."

Traumatic Albuminuria.

A transitory albuminuria may be observed following injuries to the kidneys or even after bimanual palpation of this organ. The albumin and casts may persist for a variable period with no other signs of renal involvement. In cases of movable kidney, especially during Dietl's crises, an

¹ See Grafe, Deutsch. Arch. f. klin. Med., 1914, CXVI, 328; Lund, Ugesk. f. Laeger 1915, LXXVII, 1707.

albuminuria may be noted due to obstruction in the renal circulation. In cases of ureteral stenosis, an albuminuria may be observed as the result of the impeded outflow of urine. This same type of albuminuria may be seen after blocking of the ureter by a calculus, pressure from a tumor, or twisting of the ureter.¹

Hematogenous Albuminuria.

By this type of albuminuria we have in mind one in which the albumin is excreted as a result of some alteration in the quality and quantity of the normal protein of the blood. On the other hand a distinctly hematogenous albuminuria may be the result of the excretion of an abnormal protein. This type is observed in purpura, scurvy, pernicious anemia, chronic lead or mercury poisoning, syphilis, leukemia, jaundice, cachexia, after the inhalation of anesthetics, and in diabetes.

Toxic Albuminuria.

This type is directly referable to the influence of various toxic agents upon the kidneys. The changes in the kidney may be either of a degenerative order, leading to a distinct nephritis, or may be purely circulatory. Among the substances causing such an albuminuria we find ether, chloroform, mustard, cantharides, mercury, lead, arsenic and antimony compounds, oil of turpentine, potassium nitrate and chlorate, phosphorus, carbolic acid, salicylic acid, tar compounds (aniline derivatives), petroleum and urotropin.²

Neurotic Albuminuria.

A slight transitory albuminuria may be observed in epilepsy (in which condition it may not always be found but is present invariably when marked cyanosis is seen during the attack), in apoplexy, tetanus, progressive paralysis, exophthalmic goiter, mania, delirium tremens, migraine, brain tumor, injuries to the head especially affecting the floor of the fourth ventricle, neurasthenia, and various psychoses. Not infrequently we find neurotic patients showing an albuminuria as the direct result of perverted metabolism and not as the consequence of pathologic changes in the nervous system.

Albuminuria with Definite Renal Lesions.

In acute nephritis an intense albuminuria is a constant and important symptom. The more acute the case the larger will be the amount of albumin, the elimination being generally proportionate to the severity of the disease, although some acute cases may show no albuminuria (Herringham³). The percentage of albumin varies inversely as the amount of urine, as a rule, so that it is much better to excrete a larger amount of urine with a low per-

¹ See Evans, Wynne and Whipple (Bull. Johns Hopkins Hosp., 1912, XXIII, 311), who have shown that a reflex albuminuria results from irritation of the urinary bladder.

² See Cuntz, Münch. med. Wchnschr., 1913, LX, 1656; Hydrick, Jour. Biol. Chem., 1914, XVII, p. XXXVI; Scott and Hanzlik, Jour. A. M. A., 1916, LXVII, 1838.

³ Trans. Clin. Soc. London, 1901, XXXIV, 34. See Parkinson, Brit. Jour. Child. Dis., 1916, XIII, 138; Hess, Ztschr. f. klin. Med., 1915, LXXXII, 145; Elliott, Am. Jour. Med. Sc., 1915, CL, 806; Klotz, Ibid., 827; Frothingham, Ibid., 1916, CLI, 72.

centage of albumin than a diminished amount of urine with an increased percentage of albumin. The absolute quantity of albumin excreted varies from 0.2 to 1 per cent. It may reach as high as 5 per cent. or higher, in one case of Senator being 8 per cent., but this is rare. The total excretion in 24 hours is rarely over 25 grams. Nephritis of syphilitic origin appears to be associated with the largest outputs of albumin.

In cases of active renal congestion from exposure to cold or through the action of drugs, or in chronic passive congestion due to cardiac, pulmonary, or hepatic lesions an albuminuria may be observed without any trace of an active renal lesion. The albumin, in these cases, is small in amount and runs parallel to the quantity of urine, thus differing from the excretion in true nephritis.

In chronic parenchymatous nephritis the elimination may be relatively large, exceeding, in some cases, that of the acute form.¹ In the chronic interstitial type the albuminuria is very slight, rarely amounting to more than 5 grams. In this type of nephritis the albumin may be absent at various examinations, so that frequent investigations of the urine must be made. In amyloid kidney, the urine closely resembles that of the interstitial type of nephritis, a total absence of albumin being, however, less frequently observed. The serum globulin in this type of kidney disease is relatively more increased than in any other type of renal disorder, so that the albumin-globulin quotient may be of some importance in diagnosis.

Tests for Serum Albumin.

This protein is soluble in distilled water and is coagulated by heat, if the solution be acid, at a temperature varying between 56 and 81°C. The temperature at which any protein coagulates on heating will depend upon the amount of salts present. This protein is precipitated by absolute alcohol and by salts of the heavy metals, as well as by the ordinary alkaloidal precipitants. It is levo-gyrate, its degree being represented by the following formula $(a)_D = -62.6^\circ$. It is precipitated by concentrated mineral acids, but is dissolved by somewhat large excess. With acetic acid the precipitate first formed is readily soluble in a slight excess of the acid. With concentrated alkali serum albumin forms an alkali-albuminate which is less soluble in water than is albumin, but which is soluble in an excess of the alkali. This fact accounts for the spontaneous precipitation of albumin in a concentrated urine which is alkaline in reaction.

Numerous tests have been given for the detection of albumin in the urine. The writer cannot attempt to describe all of these, but must select, therefore, those which he has found most useful.

Before any test for albumin may be made, the urine must be absolutely clear. It is advisable always to use fresh specimens, but if these are not at hand methods must be adopted to clear up the urine. In the majority of cases filtration through several folds of filter-paper will usually accomplish this. If this does not succeed, as it practically never does if the urine be

¹ See von Hösslin, *Deutsch. Arch. f. klin. Med.*, 1912, CV, 147; Cook, *Jour. Am. Med. Assn.*, 1914, LXII, 684; Williams, *Ill. Med. Jour.*, 1915, XXVIII, 186; Macris, *Grèce méd.* 1915, XVII, 17.

cloudy from the presence of bacteria, recourse must be had to precipitating agents which will carry down the suspension of bacteria.¹ Such agents are powdered magnesium oxid or carbonate, silicic acid, or saw-dust. These substances are thoroughly mixed with the urine and the mixture then filtered through double folds of filter-paper or plugs of asbestos fiber. The addition of lead acetate or any of the salts of the heavy metals is inadvisable, as the precipitates formed will include a large part of the albumin, the other precipitants not affecting the albumin directly. Occasionally the urine may be cleared by centrifugation.

It has been found that the tests for albumin are rendered more distinct if the urine be somewhat diluted. Hallauer has shown that the excess of urea and phosphates in a concentrated urine interfere to some extent with the delicacy of the reactions. As a rule, a 24-hour specimen is examined or a specimen of the urine voided in the morning and that voided at night. The variations of the voidings of the different periods of the day are occasionally quite marked, the morning specimen frequently showing no albumin while the evening specimen may show quite appreciable amounts.

Heat Test.

This test is based upon the principle that serum albumin is coagulated by heat especially in the presence of acid. One may use either acetic acid or nitric acid, but the conditions of this addition are different in each case. If acetic acid be added one must be careful lest he add an excess, as the albumin precipitate is soluble in a very slight excess of acetic acid; with nitric acid the condition is the reverse, care being taken not to add too little else the albumin will not be precipitated by the acid. A few drops of dilute acetic acid are all that is required while with nitric acid between one-twentieth and one-tenth of the volume of the urine must be added (one to two drops of 25 per cent. nitric acid per c.c. of urine).

Technic.

A test-tube is filled about three-quarters full of the clear neutral or faintly acid urine and heated by directing the flame upon the upper portion of the tube, the lower portion being held in the hand. If the fluid remains clear and the reaction is acid, no albumin is present. If a cloud is noticed it may be rendered more distinct by holding the tube against a black back-ground when the upper portion will appear more turbid than the lower. This cloud may be due to albumin or calcium phosphate, rarely to calcium carbonate. To determine which is the cause, acidulate with a few drops of 5 per cent. acetic acid. If the urine becomes clear, the precipitate first noticed is calcium phosphate; if it remains turbid and even increases in intensity of turbidity the precipitate is albumin; while if due to carbonates an effervescence will be observed. It is wise to boil the urine after the addition of each drop of acid,

¹ Vaughan (Jour. Lab. and Clin. Med., 1915, I, 55) calls attention to the erroneous practice of heating these bacteria-containing urines with alkali and filtering. As the alkali dissolves the bacterial proteins, later tests will show the presence of albumin, which was not present in the urine as voided.

so that the danger of getting an excess of acid may be more easily avoided. It is to be remembered that, if the protein be very slight in amount, and especially if the urine be originally alkaline, the protein will remain in solution owing to the formation of acid albumin. If not enough acid is added, the precipitate of phosphates may not dissolve, while if too much be added the albumin will dissolve. For these reasons it is better to add the acid after boiling. In some cases the fresh urine is already too acid to permit of coagulation, so that alkali may be added to diminish the acidity.

The presence of "nucleo-albumin" may lead to a wrong interpretation in this test. This substance is precipitated in the cold by acetic acid and may thus be differentiated. The resinous acids, which are excreted in the urine after the intake of such drugs as copaiba, cubeb, and benzoin, are not so apt to interfere with this test unless a large excess of acetic acid be used, which is never admissible.

If nitric acid be used in place of acetic acid the urine is boiled as above and concentrated nitric acid added to a strongly acid reaction. The nitric acid should never be added before boiling the urine nor should the urine be boiled after the nitric acid is added, as traces of albumin will be dissolved by the hot nitric acid. A flocculent precipitate is indicative of albumin. The phosphates and carbonates do not confuse in this reaction as they are readily dissolved. The "nucleo-albumin" is also eliminated by this test as it is readily soluble in the excess of acid. In this test the urine should be set aside and allowed to cool after the boiling is complete, as albumoses, if present, will separate out on cooling as a distinct white flocculent precipitate. A precipitate of uric acid may also form on cooling, but this is more granular and is usually colored, while the albumin precipitate is white unless an admixture of blood be present.

In case the urine be poor in salts, the tests are improved by the addition of a saturated solution of sodium chlorid. The salts hinder to a great extent the solution of the albumin by the acids. In such cases, therefore, it is wise to acidify strongly the urine with acetic acid and then add one-sixth its volume of a saturated solution of sodium chlorid as recommended by Purdy.¹ The urine is now boiled as above when a precipitate on heating will indicate albumin. The nucleo-albumin reaction is slight, the albumoses appear only on cooling, while the resinous acids may be precipitated but are soluble in alcohol, while the albumin is rendered more compact by this reagent.

Heller's Nitric Acid Test.

This test is, perhaps, more frequently employed than any other of the tests for albumin in the urine. It has a very wide field of usefulness, although it is not as delicate as some of those to be mentioned.²

A few c.c. of concentrated nitric acid are placed in a test-tube and the urine to be tested is allowed to run slowly down the side of the tube in such a

¹ Ulrich recommends the overlaying of the acidulated salt solution with urine as a contact test. Roberts uses a saturated solution of magnesium sulphate.

² See Cavazanni, *Policlinico*, 1914, XXI, 557; Cronquist, *Nord. med. Archiv.*, 1914, XLVII, 1; Möhrer, *Ibid.*, 1915, XLVIII, 1.

way as to form a distinct layer of urine above the acid. Some workers advise the addition of the acid after the urine, but if this is done it is much better practice to allow the nitric acid to flow from a pipet introduced to the bottom of the tube. The writer is always accustomed to allow the urine to flow upon the nitric acid from a long pipet so that the urine does not perceptibly mix with the acid, the tube being held at an angle of 45 degrees. Albumin, if present, is precipitated at the zone of contact in the form of a white opaque cloud or ring. This precipitate is acid-albumin which is insoluble in a slight excess of acid. A red or reddish-violet transparent ring is always obtained with normal urine owing to the reaction of the urinary pigments with the nitric acid. If the urine contains abnormal coloring matters this colored ring may assume various tints. Thus if bile be present a play of colors from red to yellow through blue or green takes place, the green being the



FIG. 81.—Conical test-glass.

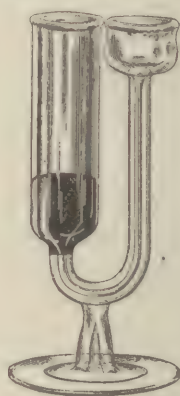


FIG. 82.—Horismascope.

characteristic coloration; if indican be in excess this colored ring may be bluish or even black; while pigments due to drugs will give colors ranging from deep red to violet. This colored ring is usually below the white opaque ring due to albumin and tends to extend down into the acid instead of up into the urine. If much nitrous acid be present in the nitric acid effervescence may be observed to such an extent that the ring of albumin may be lost. This albumin ring is usually sharply defined and separated both from the urine and acid as a white opaque ring, whose breadth will depend upon the amount of albumin in the urine. If the tube be allowed to stand for some time the ring will lose its distinct outline, a more or less diffuse cloudiness rising throughout the urine.

Albumin is, however, not the only substance to be precipitated by nitric acid under the conditions of this test. Thus we find *globulin*, *albumoses*, and *resins* precipitated exactly at the line of contact of urine and acids. If the urine be heated the albumoses will dissolve while the albumin becomes more compact. If this precipitate be due to resins the precipitate will dissolve in alcohol or ether while the albumin will remain unchanged. It is sometimes wise to shake out the urine with ether before applying this test. The globulin ring may be differentiated from that of albumin only by separating these

proteins by the method to be discussed under Serum Globulin. Globulin is usually associated with albumin, is practically never found by itself, and has practically the same clinical significance, so that for clinical purposes the differentiation of these two bodies is unnecessary. Weinberger¹ has recently shown that the addition of thymol, as a preservative of the urine, leads to the formation of a grayish-white ring just at the junction of the nitric acid and urine when this test is applied. "Below the ring there is a greenish zone extending somewhat into the acid, above it a reddish somewhat smaller zone." If this substance is suspected, the urine should be extracted by agitation with an equal volume of petrolic ether. A somewhat similar reaction has been reported by Kenney² in cases in which several drops of formalin were added as a preservative to a small amount of urine.

Besides these rings at the zone of contact, a further white or yellowish ring may be observed at this point. This ring is found in urines which are especially rich in urea and appears as a distinctly crystalline ring due to the formation of *urea nitrate*. If the urine be previously diluted this ring does not appear. If an excess of *uric acid* be present in the urine, we observe, on allowing the tube to stand for a few minutes, a distinct white ring in the urine about 1 to 2 cm. above the point of contact of the acid and urine.

If the *mucin-like bodies* previously discussed are present in slight excess, a diffuse cloud appears throughout the urine if the fluids have been slightly shaken or a distinct ring 1 to 2 cm. above the albumin ring may be observed if the urine is carefully added to the acid. This ring is seen in practically all urines, is never at the point of contact, and does not appear to have any clinical significance.

Instead of performing this test in a test-tube as described above, it may be done in a conical wine-glass, as recommended by Simon and Ogden, or may be employed as recommended by Boston. This latter worker uses a flat-pointed pipet into which is drawn from 1 to 2 inches of the urine to be tested. The exterior of the tube is then wiped perfectly dry and the pipet, with its upper end closed with the finger, is introduced to the bottom of a bottle containing pure nitric acid. By lessening the pressure of the finger the acid gradually flows up into the pipet forming a distinct line of contact between it and the urine. The same points mentioned above obtain with this test. This modification is very simple and may be recommended for general use. A further method of performing this test is the use of the horismascope (see cut). The urine is placed in the larger tube *C* and the nitric acid allowed to flow through the capillary tube *A* so that a distinct line of contact is observed. The use of this instrument frequently brings out much more clearly the albumin ring than do the other modifications.

This test is frequently employed in combination with heat. Two methods are available, either one of which may be used, although the results are somewhat different in the two cases. Some workers advise heating the nitric acid previous to the addition of the urine. This does not seem wise to the writer,

¹ Jour. Am. Med. Assn., 1909, LII, 1310.

² New York Med. Jour., 1904, LXXX, 403.

as traces of the acid albumin are undoubtedly dissolved by the hot acid and may thus escape detection. The writer is accustomed to heat only the upper portion of the tube which contains the somewhat diluted urine. In this way the urates and albumoses are thrown out of the field of action while there is much less danger of traces of albumin being dissolved. If the various points included by this test are remembered and each interfering substance removed by proper differentiation, this test is practically the most useful and reliable one for the detection of albumin in the urine.

Potassium Ferrocyanid Test.

A few c.c. of urine are strongly acidified with acetic acid and a few drops of a 10 per cent. solution of potassium ferrocyanid are added drop by drop. In the presence of albumin a faint turbidity or a flocculent precipitate will be observed, depending upon the amount of albumin present. The slow addition of the ferrocyanid is necessary, as an excess of this reagent will dissolve the precipitate first formed. In this test albumoses and nucleo-albumin are both precipitated. The former is dissolved on heating the mixture, while the latter is detected by the precipitate on the addition of the acetic acid unless the acid be added in excess. The urates do not interfere if the urine is diluted previous to making the test.

This test is recommended by many as a much more delicate one for albumin than those previously described. It reacts with a smaller amount of albumin, but it does not, in the writer's opinion, give as much general information about the urine as does Heller's test.¹

Sulpho-salicylic Acid Test.

This substance may be used either in the form of a 20 per cent. solution or in the solid state. If the solution be used it is added to the acidified urine in such a way that a distinct line of contact is formed. Albumin will be shown by a distinct white ring at the point of contact. It is preferable, however, to add to the urine a small fragment of this substance, when in the presence of albumin a turbidity or a white flocculent precipitate will be observed, depending upon the amount of albumin. The albumoses are also precipitated by this reagent, but dissolve on heating. Neither uric acid nor resins react with this substance, while the mucin-like substances are not appreciably affected.

The test, known as Roch's test, is, perhaps, the most convenient one for the use of the general practitioner, as the substance may be readily carried in the medicine case and added to the urine at the bedside.²

Spiegler's Test.

As the original reagent of Spiegler was found to be of little value in many cases, Jolles³ has modified it with the following composition: 10 grams of mercuric chlorid, 20 grams of citric acid, and 20 grams of sodium chlorid dissolved in 500 c.c. of water.

¹ See Hektoen and Retinger, *Trans., Chic. Path. Soc.*, 1919, XI, 57.

² Folin and Denis (*Jour. Biol. Chem.*, 1914, XVIII, 273) advocate this substance as a quantitative precipitant of albumin. Schall (*Münch. med. Wchnschr.*, 1920, LXVII, 164) reports that this reagent may throw down a confusing precipitate if the urine is rich in calcium. See, also, Lasch and Reitstoecker, *Ibid.*, 484.

³ *Ztschr. f. physiol. Chem.* 1912, LXXXI, 205.

The urine is acidified with acetic acid to precipitate the nucleo-albumin if present. This substance is filtered off and the filtrate superimposed by means of a pipet upon a few c.c. of the above reagent. In the presence of albumin a distinct white ring appears at the zone of contact. This reagent precipitates the albumoses which are soluble on heating. Nucleo-albumin, if present, should be removed before applying the test. In case iodids are present, mercuric iodid will be precipitated but may be removed by alcohol.

This test is the most delicate test for albumin. It shows one part of albumin in 350,000 parts of urine. This is almost too delicate for clinical work as it will show albumin in practically every specimen of urine. The writer has found this test extremely valuable in cases which showed only a faint reaction with Heller's test. If such an urine be treated with Spiegler's reagent a much more distinct albumin ring will be observed so that all doubt is thus cleared up.

Many other tests have been advocated, but the writer does not feel that they have any advantages over those outlined above. As far as delicacy of reaction is concerned Spiegler's reagent is the most delicate, the sulphosalicylic acid, heat and acid, ferrocyanid, and Heller's test following in the order named. It will thus be seen that Heller's test is the least delicate of any of the ones spoken of above, but the writer is accustomed to use it in general work, relying upon Spiegler's reagent to settle mooted points of delicacy of reaction.¹ As a general working rule it should be said that no one of the less delicate tests should be relied upon without being confirmed by some one of the others.

Quantitative Determination of Albumin.

Scherer's Method.

Fifty c.c. of urine are placed in a beaker and heated upon a water-bath. Two or three drops of dilute acetic acid are then added and the mixture boiled. A flocculent precipitate of albumin should separate out; if not, a drop or two more of acid is added until such a precipitate is obtained. The solution is then filtered through an ash-free filter which has been previously dried and weighed. The filtrate should be tested by Spiegler's reagent to see if any albumin has been dissolved. If this shows no albumin, the precipitate may then be dried on the filter-paper after being washed with water, alcohol, and ether. If the filtrate shows albumin, another test with a fresh 50 c.c. of urine must be made. Much time is saved if a larger quantity of urine be originally taken, treated as above, and small portions filtered off and tested for albumin. The addition of a small amount of a saturated solution of sodium chlorid will facilitate the precipitation of the albumin. The precipitate on the filter-paper is dried and weighed, the difference in weight between the original dried paper and that with albumin representing the amount of albumin in the 50 c.c. of urine used. The total amount is determined by a simple calculation.

¹ Leone (Policlinico, 1918, XXV, 224, sez. prat.) has introduced a test which is very delicate and should prove very reliable and satisfactory for the detection of small amounts of albumin. Leone's reagent consists of the following: Potassium bichromate, 10 grams; 100 drops of 25 per cent. sulphuric acid; 100 drops of glacial acetic acid; and 100 c.c. of distilled water. The test is made by the contact method.

Esbach's Method.

This test is carried out in a standard graduated glass tube, known as an albuminometer (see cut). This tube is filled with acidified urine to the point *U* and the Esbach reagent added to the mark *R*. This reagent consists of a solution of 10 grams of picric acid and 20 grams of citric acid in one liter of distilled water. The tube is now closed with the rubber stopper and inverted several times in order to mix thoroughly the contents. It is then allowed to stand in a test-tube rack for 24 hours, after which the amount of albumin is read off, the graduations on the tube representing the number of grams of albumin per liter of urine.

This test has many fallacies and can give only a very approximate determination of albumin. The precipitate does not settle evenly. If the albumin reads more than 4 grams per liter, the urine must be diluted to a specific gravity between 1006 and 1008 and must be kept at constant acidity. If the urine contains less than $\frac{1}{2}$ gram of albumin per liter the test is absolutely useless. Further the room temperature must be kept constant at about 15°C. or errors as high as 100 per cent. may occur. Moreover, albumoses, uric acid, creatinin, resinous acids, etc., are precipitated by this reagent.

Tsuchiya's Method.

This method¹ is a modification of the above, the reagent being a solution of phosphotungstic acid in acidulated alcohol with the following composition:

Phosphotungstic acid,	1.5 grams
Concentrated HCl,	5.0 c.c.
95 per cent. alcohol, q. s. ad.,	100.0 c.c.

This reagent is used with the Esbach tube as in the above test or in the Purdy centrifuge tube discussed later, giving far more accurate results than the Esbach test. Normal urine gives only a faint unreadable precipitate, while small amounts of albumin as well as large ones are completely precipitated by it.² This albuminous precipitate settles regularly and quickly, foaming or floating of the precipitate being rarely seen. Variations in temperature have little influence on this precipitation. It is to be remembered, however, that other urinary albuminous substances will be also precipitated. Mattice³ has shown that this method yields fairly accurate results for comparative purposes, the figures agreeing closely with those of the gravimetric methods. He has also demonstrated that the modification of this test, as introduced by



FIG. 83.—Esbach's albuminometer.

¹ Zentralbl. f. inn. Med., 1908, XXIX, 105.

² If large amounts of albumin are present, the urine must be diluted, as complete precipitation may not occur with the amount of reagent used. The supernatant fluid must be water clear before the precipitation can be regarded as complete.

³ Arch. Int. Med., 1910, V, 313. See, also, Pfeiffer, Berl. klin. Wehnschr., 1913, XLIX, 114; Semionov, Presse méd., 1914, XXII, 579; Kahn and Silberman, New York Med. Jour., 1914, C, 667.

Goodman and Stern,¹ is neither accurate nor satisfactory, largely because the point at which albumin is shown by the first turbidity with this reagent (and hence the amount of albumin reacting) varies markedly with the dilution of the urine.

The writer advises, therefore, that this method of Tsuchiya be used for reliable comparative clinical results, the gravimetric or Kjeldahl methods being applied for scientific purposes. In case the latter method be used, multiply the nitrogen values of the precipitated and washed albumin by 6.3 to obtain the albumin values.

It is perhaps unnecessary to state at this point that in speaking of per cent. of albumin, one should have reference only to the number of grams of albumin by weight in 100 c.c. of urine. The urine very rarely contains more than 5 per cent. of albumin, although Salkowski has reported a case in which 8 per cent. was observed, the albumin separating out as a white amorphous precipitate on standing. This fact should be remembered, as the appearance of such a precipitate in the untreated urine might be very misleading.

Purdy's Centrifugal Method.

To 10 c.c. of the urine placed in a centrifuge tube, 3 c.c. of a 10 per cent. solution of potassium ferrocyanid and 2 c.c. of 50 per cent. acetic acid are added. The reagents and urine are then mixed by placing the thumb over the end of the tube and inverting it several times, after which the tube is allowed to stand for 10 minutes. It is then placed in a centrifuge the radius of which, with its tubes extended, must be $6\frac{3}{4}$ inches. The tubes are revolved for exactly three minutes at a uniform speed of 1,500 revolutions per minute. The amount of albumin is then read off in bulk percentage, each division of the tube representing 1 per cent., as only 10 c.c. of urine are used and the divisions represent tenths of a c.c. One per cent. by bulk represents 0.021 per cent. by weight of albumin.

This method is very satisfactory, although not absolutely accurate. It is difficult to keep a centrifuge running uniformly at the above rate, unless a speed indicator be watched during the entire period. The method is more exact and more expeditious than the Esbach method and is to be recommended, therefore, for clinical estimations of the amount of albumin.²

Removal of Albumin.

It is advisable in many of the general quantitative tests applied to the urine that the albumin should be removed if it is present in more than traces. Usually this may be done by acidifying with acetic acid and boiling until the precipitate is flocculent. The filtrate in such cases will usually be clear and contain no albumin. As this test does not eliminate the albumoses and further as the boiling acid may hydrolyze a small amount of the albumin into albumoses, Hofmeister recommends the following method. Ten c.c. of a 40 per cent. solution of sodium acetate and the same amount of 10 per cent.

¹ Jour. Am. Med. Assn., 1908, LI, 2055. Autenrieth and Mink (Münch. Med. Wchnschr., 1915, LXII, 1417), Claudius (Hospitalstid., 1916, LVIII, 1237) and Marshall, Banks and Graves (Arch. Int. Med., 1916, XVIII, 250) have advanced colorimetric methods for the determination of albumin.

² See Strzyzowski, Ztschr. f. physiol. Chem., 1913, LXXXVIII, 25.

ferric chlorid are added to the urine when it will be colored a bright red. The urine is neutralized or rendered very faintly acid and is then boiled. The albumin separates out along with the basic ferric acetate and is filtered off. This method is not applicable if glucose is present.¹

(b) Serum Globulin.

Serum globulin is associated in the blood with serum albumin. This protein is not a single body, but is probably a mixture of two forms known as euglobulin and pseudo-globulin. These two fractions differ in their precipitation and solubility constants and must be looked for in works on physiologic chemistry. The former of these probably occurs in most urines, constituting a large part of what has been called "nucleo-albumin" (see above).

Serum globulin, in the specific sense, is usually found in the urine in every case in which serum albumin is observed. Cases are reported in which each one of these protein bodies has appeared separately, but this is not the normal finding. Its excretion as compared with that of serum albumin varies from 10 to 75 per cent. of the total protein. The relation of the albumin to the globulin of the blood is as 1.5 is to 1. This relation, known as the "*albumin quotient*," is by no means the same in the urine. In cases of amyloid degeneration of the kidney the globulin may be very much increased beyond that observed in other chronic affections of the kidney, the albumin quotient being usually lower than one. Senator considers this an important point in the diagnosis of this condition. In the various types of nephritis we find the globulin being greater the more acute the condition, so that in acute diffuse nephritis the albumin quotient may be very low, while in chronic parenchymatous nephritis the quotient ranges from two and five-tenths to five and five-tenths. As the nephritis improves the relative amount of globulin diminishes, but increases with each acute exacerbation.²

Globulin is insoluble in water, but soluble in dilute solutions of sodium chlorid, dilute acids, or alkalies, unless these be exceedingly dilute and their action not prolonged. If, therefore, urine containing globulins be highly diluted with water the globulin will be precipitated in the form of a distinct cloud. The precipitation constants with various inorganic salts must be learned from text-books on physiologic chemistry.

Qualitative Test for Globulin.

The urine is rendered alkaline by the addition of a few drops of ammonium hydrate and the precipitated phosphates filtered off. To the filtrate is then added an equal volume of a saturated solution of ammonium sulphate, the mixture is allowed to stand one hour and is then filtered. The albumoses and nucleo-albumin may also be precipitated in this way. Ammonium urate does not usually separate out in the hour. The precipitate on the filter is then washed with a half-saturated solution of ammonium sulphate until the filtrate is albumin-free. A distinct precipitate is usually evidence of the presence of

¹ See Tracy and Welker (Jour. Biol. Chem., 1915, XXII, 35) for the use of aluminium hydroxide cream in removing albumin.

² Belfield (Jour. Am. Med. Assn., 1915, LXIV, 2040) calls attention to the presence of globulin, with or without albumin, in conditions associated with leakage of the seminal vesicles.

globulin, as the albumin is not precipitated until the urine is completely saturated with the ammonium sulphate. In order to eliminate the other factors the precipitate is dissolved in water and heated on a water-bath to coagulate the proteins. The solution is filtered, the precipitate washed with water and heated on a water-bath with a 1 per cent. solution of sodium carbonate. It is then filtered and neutralized with acetic acid. If a precipitate occurs it is globulin, as the albumoses and nucleo-albumin would not be precipitated by such treatment.

Paton advises the use of a contact method in detecting globulin. The phosphates are removed as mentioned above and the filtered urine allowed to run down the side of a test-tube containing a few c.c. of a saturated solution of sodium sulphate. A white ring will indicate the presence of globulin.

Quantitative Determination.

The phosphates are removed as above and 100 c.c. of the clear filtered urine are treated with an equal volume of a saturated solution of ammonium sulphate or directly saturated with magnesium sulphate. The precipitated globulin is collected on a dried and weighed filter and washed with a half-saturated solution of ammonium sulphate in the former instance or with a saturated solution of magnesium sulphate in the latter case. The final washings should show no trace of a reaction for albumin. The funnel with the filter-paper and its contents are then dried at 110°C. The ammonium sulphate is washed from the precipitate with hot water and the precipitate is then dried with alcohol, ether, and finally at 110°C., until the weight becomes constant. The difference between the weight of the filter-paper and the filter-paper plus the globulin gives the amount of globulin in 100 c.c. of urine.

(c) Proteoses.

These are intermediate products of the digestion of protein by ferments, by acids, or by bacteria. In the normal digestion we find the products passing through the following stages: protein, acid albumin, primary proteoses (of which there are two, namely, protalbumose and heteroalbumose), secondary proteoses (the only well-established representative being deuteroalbumose), peptone, amino-acids and hexone bases. The proteoses and peptones are very soluble diffusible bodies which are not coagulated by heating. The primary proteoses are precipitated by half-saturation with ammonium sulphate, the secondary proteoses are precipitated only after complete saturation with this salt, while the peptones are not precipitable in either one of these ways. The primary proteoses are precipitated by nitric acid, thus differing from the secondary types which are not so precipitated. In the urine we find representatives of both types of proteoses, while more or less doubt exists as to whether true peptone has ever been isolated from the urine.

Primary Proteoses.

Bence-Jones' Protein.

This body was first discovered by Bence-Jones¹ and was regarded as a heteroalbumose. Later work by Magnus-Levy² has shown that it is in all

¹ Med. and Chir. Trans., 1850, XXXIII; Phil. Trans. Royal Soc., 1848, I, 55.

² Ztschr. f. physiol. Chem., 1900, XXX, 200.

probability a true albumin, as its digestion products include protalbumose which could hardly be derived from true heteroalbumose. As the exact chemical nature of this body has not been definitely settled, it will be discussed under the above heading, although it probably does not properly belong there. This protein must be regarded as *sui generis*. Hektoen has been able to prepare a specific precipitin for it and Bayne-Jones and Wilson have shown, by precipitin, complement fixation and other immunological reactions, unquestionable differences between it and the proteins of normal serum. This body differs from all other types of protein material which occur in the urine in its property of precipitating when heated to as low a temperature as 40°C. and of practically completely dissolving on boiling, to appear again on cooling. A second characteristic of this body is the readiness with which it dissolves in dilute ammonia after it has been precipitated with alcohol. The excretion of this body in the urine has been called "heteroalbumosuria," "myelopathic albumosuria of Bradshaw," "Kahler's disease" and "Bence-Jones' albumosuria."

The amount of this protein body excreted is somewhat variable. In Bence-Jones' original case an output of 6.7 per cent. or a total amount of 70 grams in the 24 hours was observed, while Coriat¹ reports a case in which none was found in the urine, although 4 per cent. was present in the pleuritic effusion. Between these limits we find the majority of cases showing an output usually not over 1 per cent. The literature contains about 35 cases showing the excretion of this body. The output of this body appears to be constant during the day and not affected in any way by the diet. Little is known regarding the direct origin of this body.² It undoubtedly has some association with the bone-marrow, but just what is not clear. "We may imagine, however, that through the agency of the cells of the abnormal tissue, that is their products of metabolism, the normal transformation of the ingested albumin into tissue-albumin is impeded, resulting in the production of the substance in question, which is then eliminated as foreign matter" (Simon).

This body is excreted in cases associated with the occurrence of multiple myelomata of the bones, especially when these affect the thoracic skeleton. It has been observed in a very few cases of leukemia and in a case associated with metastatic carcinoma.³ However, these findings are so rare that the

¹ Am. Jour. Med. Sc., 1903, CXXVI, 631.

² Rosenbloom (Arch. Int. Med., 1912, IX, 236 and 255) advances the possibility of osseolumoid as a precursor of this protein. See, also, Hopkins and Savory, Jour. Physiol., 1911, XLII, 106; Cathcart and Henderson, Jour. Path. and Bacteriol., 1912, XVII, 238.

³ Boggs and Guthrie, Am. Jour. Med. Sc., 1912, CXIV, 803; Bull. Johns Hopkins Hosp., 1912, XXIII, 353; Ibid., 1913, XXIV, 368; Folin and Denis, Jour. Biol. Chem., 1914, XVIII, 277; Schütz, Deutsch. Arch. f. klin. Med., 1914, CXIII, 441; Sexsmith and Klein, Med. Record, 1915, LXXXVIII, 600; Schumm and Kimmerle, Ztschr. f. physiol. Chem., 1914, XCII, 1; Martini, Policlinico, 1915, XXII, 451; Groat and Brewer, Jour. Lab. and Clin. Med., 1916, I, 805; Taylor and Miller, Jour. Biol. Chem., 1916, XXV, 281; Taylor, Miller and Sweet, Ibid., 1917, XXIX, 425; Hermann and Wilson, Lancet, 1918, II, 870; Abderhalden, Ztschr. f. physiol. Chem., 1919, CVI, 130; Ide, Bull. acad. roy. méd. Belg., 1920, XXX, 914; Decastello, Wiener Arch. f. inn. Med., 1920, I, 335; Thannhauser and Krauss, Deutsch. Arch. f. klin. Med., 1920, XCXXIII, 183; Wallgren, Hygiea, 1921, LXXXIII, 1; Walters, Med. Record, 1921, C, 847; Jour. Am. Med. Assoc., 1921, LXXVI, 641; Hektoen, Ibid., 929; Speares, Dublin Jour. Med. Sc., 1921, Ser. 4., 193; Oftedal, Jour. Am. Med. Assoc., 1921, LXXVII, 1547; Bayne-Jones and Wilson, Bull. Johns Hopk. Hosp., 1922, XXXIII, 37 and 119.

presence of this urinary protein may be regarded as practically pathognomonic of multiple myelomata. In some cases of this disease the urine does not show the Bence-Jones' body, so that a negative finding does not necessarily preclude the condition. Ellinger has shown that this disease may take its course without the occurrence of local bone symptoms, but may be associated with a marked anemia. It is, therefore, wise in cases of obscure anemia to test the urine repeatedly for this body.

Tests for Bence-Jones' Body.

The specific reaction for this protein is observed on heating the acidified urine very slowly.¹ At a temperature varying from 50° to 60° a slight cloud changing to a marked turbidity and then into a dense cloud will be observed. This may be so intense that the urine appears distinctly milky. This turbidity may change into a heavy sticky precipitate or coagulum as the temperature approaches the boiling-point. When the boiling-point is reached the precipitate entirely or partially dissolves, especially if the boiling be continued from one to three minutes. The precipitate may not absolutely all dissolve on boiling, as variations in the acidity of the urine and the amount of mineral salts present may affect this process. If the tube be allowed to stand after being boiled the precipitate returns as the fluid cools. None of the other protein bodies give this sequence of precipitation, dissolving, and reprecipitation. Hugounenq suggests the name "*thermolytic albuminuria*" for the excretion of this body in the urine.

Upon the addition of concentrated nitric acid, drop by drop, a temporary turbidity develops which disappears on shaking, but persists if more acid be added. If the mixture be heated the precipitate will dissolve and reappear on cooling. This same reaction may be observed on applying any of the tests for serum-albumin outlined above.

This protein is precipitated from its solution by the addition of two volumes of a saturated solution of sodium chlorid to urine which has been previously acidified with acetic acid. The addition of two volumes of saturated solution of ammonium sulphate likewise causes its complete precipitation. It may be then washed with alcohol and ether and dried over sulphuric acid.

Boston² has proposed the following test for this body. Fifteen to twenty c.c. of filtered urine are placed in a test-tube and mixed with an equal volume of a saturated solution of sodium chlorid, the tube being shaken to insure a thorough mixing of the fluids. Two or three c.c. of a 30 per cent. solution of sodium hydrate are added and the mixture vigorously shaken. The upper one-fourth of the mixture is then gradually heated to the boiling-point and a solution of 10 per cent. lead acetate added drop by drop, the heating being continued after each addition. When the drop of lead solution comes in contact with the liquid a copious pearly or creamy cloud appears at the surface, becoming less dense as the boiling-point is neared; and when ebullition is prolonged for from one-half to one minute the upper portion of the liquid

¹ Miller and Sweet (Jour. Biol. Chem., 1921, XLVIII, 21) state that only fresh urine should be used for this test as changes may occur due to the proteolytic enzyme of the urine.

² Clinical Diagnosis, Philadelphia, 1905.

shows slight browning, which deepens to a dull black color. Standing intensifies the reaction, and if this be prolonged for several hours the black precipitate falls through the clear stratum of liquid, collecting in the bottom of the tube as a coarsely granular pigment. This reaction is based upon the fact that this body contains a large proportion of loosely bound sulphur. Lindemann¹ finds that this body really contains no more such sulphur than does serum albumin, while Wood could obtain no more blackening than with other proteins. This test would seem to have little value as it is in no way distinctive for the Bence-Jones' protein.

The quantitative determination of this body may be made by precipitation with two volumes of saturated solution of ammonium sulphate and the washing and drying of this precipitate as previously mentioned. The Tsuchiya method is useful as an approximate estimation in the absence of albumin.

Secondary Proteoses.

Deuteroalbumose is probably the body which has been found in the urine in cases in which peptone was reported. This secondary proteose differs in its reactions from the primary proteoses and the Bence-Jones' protein. It is precipitated only on complete saturation of the urine with ammonium sulphate. In performing this test the urine should be made albumin-free, preferably by the Hofmeister method previously discussed. Nucleo-albumin may be precipitated by basic lead acetate. Urine containing deuteroalbumose does not become cloudy on boiling; does not regularly give Heller's test, but does react with the ferrocyanid test when the neutral salts are present in fairly large quantities, and reacts in the cold with sulpho-salicylic acid and with Spiegler's reagent, but the precipitate dissolves on heating to reappear again on cooling. This reaction with the latter reagents in the cold may be distinguished from that of albumin by boiling the mixture and filtering while hot. The albumin remains on the filter while the albumose is present in the filtrate, appearing as a distinct precipitate as the solution cools.

Tests for Albumoses.

To a few c.c. of urine, from which albumin and nucleo-albumin have been removed as outlined above, add one-fifth its volume of concentrated acetic acid. A 10 per cent. solution of phosphotungstic acid is then added, when the urine remains clear on standing if albumoses are absent; while a milky turbidity is observed in 5 to 10 minutes if these substances are present. If this precipitate be filtered off (warming will facilitate the clumping of the precipitate), washed with distilled water and then dissolved on the filter with a very dilute solution of sodium hydrate, the solution will have a distinctly blue color. This solution is warmed until it becomes clear, more sodium hydrate being added if necessary. It is then cooled and the biuret test applied by adding a few drops of strong sodium hydrate and a few drops of dilute (2 per cent.) copper sulphate solution. On warming this mixture a beautiful red color will be observed. *Fittipaldi*² advises that 10 c.c. of urine be treated with 60 c.c. of absolute alcohol and allowed to stand until the next

¹ Deutsch. Archiv. f. klin. Med., 1904, LXXXI, 114.

² Gazz. d. osp., 1911, XXXII, 515; Deutsch. med. Wchnschr., 1921, XLVII, 42.

day. The alcohol is carefully poured off, the precipitate is dissolved in the least possible amount of 30 per cent. NaOH and this alkaline solution is treated with a few drops of a freshly prepared ammoniacal nickel solution.¹ In the presence of albumose or peptone an orange-red coloration appears. This test seems reliable and does not react with albumin.

Bang's Method.

Ten c.c. of urine are heated in a test-tube with 8 grams of finely powdered ammonium sulphate until the salt has been dissolved. The mixture is boiled for a few seconds and is then centrifugalized for one-half to one minute. The supernatant fluid is then poured off and the precipitate extracted with alcohol to remove urobilin. After pouring off the alcohol the residue is dissolved in a little water, the solution is boiled to remove albumin and filtered. The filtrate is shaken out with chloroform to remove any traces of urobilin which may have escaped previously. The watery solution is then poured off from the chloroform and tested as above for the biuret reaction.

Clinical Significance.

Deuteroalbumose may occur in the urine either alone or associated with albumin. It is observed in a great variety of conditions, so that distinct types of albumosuria may be noted.

Large accumulations of pus anywhere in the system lead to the excretion of deuteroalbumose as a result of the breaking down of the pus cells and the later absorption of the hydrolyzed material. This form, known as *pyogenic albumosuria*, is observed in pneumonia during the stage of resolution, in gangrenous processes anywhere in the system, in empyema, bronchiectasis, abscess formation, and in epidemic cerebrospinal meningitis. In this latter condition the differential diagnosis from a tubercular meningitis often rests on the appearance of albumose in the urine.

A *hepatogenous* form of albumosuria occurs in any condition associated with marked disturbance of hepatic function, as for instance in acute yellow atrophy, phosphorus poisoning, cirrhosis, carcinoma, and catarrhal jaundice. Little is known of the origin of the albumose in this condition.

An *enterogenous* type is observed in cases of gastric or intestinal ulcer, whether the latter be due to typhoid fever or dysentery, while intestinal tuberculosis is less frequently associated with the appearance of albumose in the urine. In these cases the breaking down of the tissues may be responsible for increased absorption both of the products of hydrolysis of the tissues and of the food.

An albumosuria of *hematogenous* origin has been observed in cases of scurvy, leukemia, purpura, dermatitis, poisoning with hemolytic agents, pregnancy, especially after the death of the fetus, and in various psychoses, as well as in carcinoma affecting any part of the system. The albumosuria in these cases is probably referable to the increased lysis of the cells under the influence of the exogenous or endogenous toxins.

¹ A 5 per cent. solution of nickel sulphate mixed with an equal volume of ammonia water.

A *febrile* type is observed in practically all fevers, more especially the infectious types, such as measles, scarlet fever, diphtheria, acute articular rheumatism, smallpox, and mumps. This is referable both to the influence of the toxins of the disease in producing increased protein disintegration as well as to the associated septic conditions.

A large number of other conditions are associated with the appearance of albumose in the urine. Such a state is due to the breaking down either of tissue or of an exudate, and may, therefore, appear in almost any type of disease. In some cases albumose may appear in the urine, following the ingestion of a large amount of albumose. This is the *digestive* or *alimentary albumosuria* and appears to be indicative of an ulcerative condition somewhere along the intestinal tract. Pollak¹ shows that this albumosuria may be due, in part at least, to pathological conditions of the kidney, and suggests the name *renal albumosuria* for such cases.

As previously stated, albumose may be associated in the urine with albumin, constituting the *mixed albuminuria* of Senator. In these cases the albumosuria may precede the albuminuria, may alternate with it, or continue after it has disappeared. This condition is particularly prominent in cases of nephritis, especially of the syphilitic type, and should be watched with care. In any case of albumosuria it is necessary to exclude contaminations with foreign material, especially with spermatic or prostatic secretions.

(d) Peptone.

True peptone rarely if ever appears in the urine. Peptones are the last hydrolytic products of protein which give the biuret reaction. This body is not precipitated on saturation with ammonium sulphate as are the other types of protein material. Ito reports the finding of true peptones in the urine in cases of croupous pneumonia, pulmonary tuberculosis, ulcer of the stomach, and in women during the puerperal period. In these cases deutero-albumose was also present, so that there is a possibility that the peptone isolated by Ito was derived from the urinary albumose. Many reports of peptonuria are found in the literature, but the substance dealt with in practically all of these cases was probably some type of albumose and not true peptone.

(e) Hemoglobin.

This body is the normal coloring matter of the blood and is to be regarded from the chemical standpoint as a chromoprotein. In the normal metabolism disintegration of red blood-corpuscles is constantly occurring, but this is not sufficient to lead to a hemoglobinemia and a resulting hemoglobinuria. These two conditions must go hand in hand, the latter being impossible without the former.

When the destruction of the red cell becomes so extensive that the accumulation of the blood pigment in the blood-current is so great that the liver is unable to convert it into bilirubin, hemoglobinemia and hemoglobinuria must result.² While the distinct limit of destruction of cells necessary to

¹ Ztschr. f. d. ges. exper. Med., 1914, II, 314.

² See Wilbur and Addis, Arch. Int. Med., 1914, XIII, 235; Addis, Ibid., 1915, XV, 413. Haessler (Jour. Exp. Med., 1922, XXXV, 515) shows that flood diuresis so far lowers the renal threshold for hemoglobin that the pigment appears as a result of a hemoglobinemia, insufficient to lead ordinarily to even a trace.

produce this condition is not definitely settled, it may in general be said to occur when approximately one-sixtieth of the hemoglobin of the corpuscles is set free. The protein really excreted in the urine is not hemoglobin, but methemoglobin, so that the term methemoglobinuria would be better used, although a direct hemoglobinemia does obtain.

From what has been said above it is evident that the excretion of this protein will inevitably occur after the use of the so-called hemolytic poisons. Among these we find ether, chloroform, snake-venom, arseniuretted hydrogen, phosphorus, hydrogen sulphid, toluylendiamin, mushrooms, anilin, lactophenin, bile salts, chlorates, pyrogallie acid, naphthol, carbolic acid, carbon monoxid, and tuberculin. Hemoglobin will appear in the urine in cases of poisoning with the above substances only when the hemoglobinemia is extensive. In mild cases, the liver will be called upon to form increased biliary pigment and the urine will, therefore, contain bile pigments instead of blood pigments. Likewise, we find a hemoglobinuria following transfusion of the blood of animals into man, after severe burns, exposure to cold, in the course of any of the specific infectious diseases, and in malaria¹ and syphilis. The so-called "black-water fever" is more probably a malarial hematuria than a hemoglobinuria.

A paroxysmal type of hemoglobinuria has been occasionally reported in the literature. This occurs in typical paroxysmal forms after exposure to cold or exertion, and is often preceded by a typical "infectious" onset, such as chill, fever, and malaise, along with pain in the lumbar region. The hemoglobin may be excreted for several days and then disappear with no untoward symptoms. This condition is very rare and its cause uncertain.² An epidemic hemoglobinuria occurs at times in the new-born and is associated with a distinct hemoglobinemia, jaundice, and cyanosis.

The urine, in cases of hemoglobinuria, may be clear, but is generally turbid, and varies in color from a bright red to almost a black. The turbidity gives the appearance of a peculiar smoky or hazy urine. The urine must be examined when freshly voided, as blood-corpuscles soon disintegrate in the urine, giving it the same appearance as noted in hemoglobinuria. The clinical significance of hematuria and hemoglobinuria are much different and should not be confounded. If the urine be centrifuged the supernatant fluid will be a clear blood-colored liquid and the sediment will show none or very few red cells.

¹ Urriola (Interstate Med. Jour., 1912, XIX, 74) claims that the black urinary pigment of the urine is pathognomonic of malaria.

² According to Cooke (Am. Jour. Med. Sc., 1912, CXLIV, 203), syphilis is the most important, if not the only, etiologic factor in paroxysmal hemoglobinuria. See, however, Lindbom, Hygiea, 1913, LXXV, 885; Ztschr. f. klin. Med., 1913, LXXIX, 147; Young, Jour. Am. Med. Assn., 1914, LXII, 356; Widal, Abrami and Brissaud, Sem. Méd., 1914, XXXIII, 613; Porges and Strisower, Deutsch. Arch. f. klin. Med., 1914, CXVII, 13; Dennie and Robertson, Arch. Int. Med., 1915, XVI, 205; Daniels, Nederl. Tijdschr. v. Geneesk., 1915, II, 753; Sabroe, Hospitaltid., 1915, LVIII, 1056; 1056; Gasharrini, Policlinico, 1915, XXII, 1537; Hintze, Deutsche Med. Wchnschr., 1916, XLII, 1186; Johannessen, Norsk Mag. f. Laegevidensk., 1917, LXXVIII, 179; Schiassi, Policlinico, 1920, XXVII, 346 and 397; Datta, Ibid., 422; Weinberg, Münch. med. Wchnschr., 1921, LXVIII, 422; Salen, Hygiea, 1921, LXXXIII, 440 and 497; Thornhill, New Orleans Med. & Surg. Jour., 1921, LXXIV, 224; Burmeister, Ztschr. f. klin. Med., 1921, XCII, 19; Salom, Gaceta Med. de Caracas, 1921, XXVIII, 328; Kaznelson, Deutsch. Arch. f. klin. Med., 1921, CXXXVIII, 46; Jones and Jones, Arch. Int. Med., 1922, XXIX, 669.

The tests for the presence of blood pigments must be applied in order to differentiate this protein from the other types. Naturally, any specimen of urine containing hemoglobin will react to the albumin tests previously given so that it may be very difficult to determine whether a true albuminuria is coexistent. As a rule, in such conditions there is an associated nephritis so that all the findings of this latter condition may obtain. The chemical tests indicate the presence of hemoglobin or of any of its derivatives and do not differentiate a hemoglobinuria from a hematuria. Microscopic examination for the presence of red blood-cells is the only possible way of clearing up such a diagnosis. A spectroscopic examination of the urine will differentiate the types of blood pigments (see Blood).

Tests for Hemoglobin and Derivatives.

Heller's Test.

A few c.c. of urine are strongly alkalized with sodium hydrate and heated. Either at once or on standing a brownish-red precipitate of the phosphates and the carbonates of the alkaline-earths is formed, the color being due to the hematin carried down by the phosphates if blood is present. If the urine contains a large amount of foreign pigments, this red coloration may not be easily noted. In this case filter off the precipitate and dissolve it in acetic acid, when the solution becomes red if blood pigment is present, the color gradually fading upon exposure to air. If this test be controlled by the spectroscopic tests for hematin in alkaline solution it becomes quite reliable and very delicate. It indicates one part of oxyhemoglobin in 4,000 of urine.

Donogany's Test.

Ten c.c. of urine are treated with 1 c.c. of ammonium sulphid solution and 1 c.c. of pyridin. If blood be present, the urine will assume a more or less intense orange color, which may be more evident on looking through the test-tube lengthwise. In this case the hemoglobin has been converted into hemochromogen, which may be recognized by the spectroscopic test. This test is more delicate than the previous, showing one part of blood to 8,000 of urine. Instead of the above tests, which are more directly applied in urine, the various tests as discussed under Feces may be applied.

The spectrum of the various blood pigments will be discussed later, so that the writer need only refer to the section on Blood for this. If the urine contains fresh blood the spectrum is that of oxyhemoglobin, while in cases of hemoglobinuria or of hematuria of renal origin the spectrum is that of methemoglobin. The urine to be tested spectroscopically should be slightly acid and perfectly clear. If a large amount of blood pigment be present, the spectrum will be much clearer if the urine be diluted. This dilution should not be carried too far, otherwise the absorption lines will not appear. In testing for methemoglobin the spectrum of neutral as well as of alkaline methemoglobin should be looked for.

(f) Fibrin.

The occurrence of this protein in the urine is very rare.¹ As fibrin is derived from fibrinogen through the action of the fibrin ferment, the presence of

¹ See O'Connor, *Am. Jour. Med. Sc.*, 1920, CLIX, 729.

the former body presupposes that the latter two substances have been present somewhere along the genitourinary tract. This substance is an elastic, grayish, stringy material insoluble in water and alcohol. Chemically, fibrin belongs to the group of globulins; it is soluble with difficulty in dilute saline solutions, is coagulated by heat, and precipitated either by great dilution with water or by saturation with magnesium sulphate.

This protein may occur in the urine either in the coagulated form or in solution. It is found in any condition in which large amounts of blood are present in the urine, whether the blood comes from the kidneys or points below. It may coagulate immediately after voiding or may occur as preformed clots which are formed either in severe inflammations of the pelvis of the kidney, of the ureter, bladder, or urethra. It occurs also in cases of chyluria and rarely in direct nephritis.

In some cases the fibrin is in solution, especially in urines containing no blood. This fibrin separates out in the form of a coagulum on standing or may change the urine into a distinctly gelatinous mass. This so-called "spontaneously coagulable urine" is seen more frequently in cases of chyluria, but may be observed in rare cases of nephritis.

Test for Fibrin.

The clotted material is filtered off from the urine, is thoroughly washed with water and boiled in a 1 per cent. solution of sodium carbonate. On cooling, this solution may be tested as outlined under Serum Albumin.

(2) Carbohydrates.

Normally the urine contains traces (0.01 to 0.03 per cent.) of carbohydrates which are incapable of detection by the ordinary clinical tests. Besides these true carbohydrates the urine contains other substances which react, especially toward copper solutions, as do the monosaccharides. These latter reducing bodies are uric acid, creatinin, conjugated glycuronic acids, and various pigments, either normal ones excreted in unusual amounts or abnormal ones excreted in usual amounts. The total output of the reducing bodies of the normal urine varies between 2 and 3 grams in 24 hours, while the normal true carbohydrates of the urine vary from 0.2 to 1 gram per diem. Benedict proposes the name glycuressis instead of glycosuria, for this normal sugar excretion which requires very delicate tests for its detection.

(a) Glucose (d-Glucose) $(\text{CH}_2\text{OH}-(\text{CHOH})_4-\text{CHO})$.

The normal blood contains about one part per thousand of glucose. Whether this sugar is in the free state or in combination with other molecules, as for instance as the so-called jecorin of Drechsel, is at present an unsettled question. According to Claude Bernard, sugar will appear in the urine whenever more than three parts per thousand are present in the circulating blood. This figure is, in view of the recent work of von Noorden, Stern, and Liefmann, much too high, as the average finding in their cases was 0.85 part per thousand. The excretion of sugar in the urine is known as glycosuria and presupposes an excess of sugar in the blood (hyperglycemia). In only one form of glycosuria do we find absence of this hyperglycemia; that is, in con-

ditions in which the kidneys become less impervious than normally to the sugar circulating in the blood. This type of glycosuria is most frequently observed in cases of poisoning with phloridzin and, also, after intravenous injection of large quantities of physiologic salt solution (the so-called "salt glycosuria"), and has led to the assumption of the clinical entity "*renal diabetes mellitus*." The pathology of such a condition is little understood, so that we may for the present disregard this type and limit our discussion to the glycosuria which inevitably follows a hyperglycemia.¹

Glycosuria.

The normal metabolism is such that any excess of carbohydrate food is converted, up to a certain point, into glycogen and stored up in the liver. Should this ingestion of carbohydrates exceed the functional power of the liver to convert it into glycogen, the excess will pass through the hepatic filter into the circulating blood, thus causing directly a hyperglycemia. Unless increased muscular activity is sufficient to utilize this excess,² the kidneys will excrete sugar until the normal relations again obtain. This is the purely alimentary type of glycosuria and ceases as soon as the intake is diminished. The type of food ingested has much to do with the extent of the glycosuria. Under normal conditions it matters relatively little how much carbohydrate is ingested in the form of starch, as the products of hydrolysis are gradually absorbed and do not lead to overactivity of the liver with a resulting hyperglycemia and glycosuria. On the other hand, a certain limit, different for each individual, is observed in the amount of sugar which may be ingested without causing a glycosuria. For this reason Naunyn has regarded alimentary glycosuria as of two distinct types: (1) that following the ingestion of starch, which he styles *glycosuria ex amylo*, and (2) that following the ingestion of an excess of sugar, *glycosuria e saccharo*. The amount of starchy or of saccharine food which a person may ingest without a glycosuria is known as the *assimilation limit* or *degree of tolerance* for such food. This factor varies for each individual under normal conditions and under pathologic influences is dependent

¹ See de Langen, Nederl. Tijdschr. v. Geneesk., 1913, II, 1454; Ferannini, Riforma Med., 1914, XXX, 232; Landau, Rev. de Med., 1914, XXXIV, 145; Strouse and Beifeld, Jour. Am. Med. Assn., 1914, LXII, 1301; Salomon, Deutsch. med. Wchnschr., 1914, XL, 217; Galambos, Ibid., 1301; Gram, Hospitalstid., 1915, LVIII, 329; Lewis and Mosenthal, Bull. Johns Hop. Hosp., 1916, XXVII, 133; Murlin and Niles, Am. Jour. Med. Sc., 1917, CLIII, 79; Beard and Grave, Arch. Int. Med., 1918, XXI, 705; Goto, Ibid., 1918, XXII, 96; Langstroth, Am. Jour. Med. Sc., 1919, CLVII, 201; Bailey, Ibid., 221; Allen, Wishart and Smith, Arch. Int. Med., 1919, XXIV, 523; Galambos, Deutsch. med. Wchnschr., 1920, XLIV, 600; Naito, Tohoku Jour. Exp. Med., 1920, I, 131; Paullin, Jour. Am. Med. Assoc., 1920, LXXV, 214; Strouse, Arch. Int. Med., 1920, XXVI, 768; Frank and Nothmann, Münch. med. Wchnschr., 1920, LXVII, 1433; Nürnberger, Deutsch. med. Wchnschr., 1921, XLVII, 1124; Marsh, Arch. Int. Med., 1921, XXVIII, 54; Lewis, Ibid., 1922, XXIX, 418; Nash, Jour. Biol. Chem., 1922, LI, 171; Seitz and Jess, Münch. med. Wchnschr., 1922, LXIX, 6; Uedinghoff, Klin. Wchnschr., 1922, I, 126; Labbé, Bull. de la soc. méd. des Hôp. de Paris, 1922, XLVI, 198; Leyton, Practitioner, 1922, CVIII, 113; Labbé, Ann. Med., 1922, XI, 273.

² It must be remembered, however, that a certain portion of this sugar excess is reduced to fat and stored up in the tissues. Peirce and Keith (Proc. Soc. Exper. Biol. and Med., 1915, XII, 210) believe that the kidney normally oxidizes a certain amount of glucose and that sugar normally gains entrance into the kidney cells in proportion to its concentration in the blood, the amount oxidized in these cells depending on the sugar concentration.

upon the state of the intestines, liver, pancreas, muscles, and kidney. A normal person may stand an intake of from 200 to 300 grams of glucose without excreting more than traces in the urine, but in the majority of persons this figure would, perhaps, be found to be more nearly 150 grams than 300.¹ It has been found that the administration of as small amounts as 50 grams of galactose and lactose was followed by an excretion of these sugars in the urine, while maltose, dextrose, levulose, and saccharose required much larger intakes. The excreted sugar, following the increased ingestion, is in most cases similar to that taken in, although Moritz has shown that some of the polysaccharides may be partially hydrolyzed into their monosaccharide components. It has been found by Worm-Müller that a large intake of cane-sugar is not followed by a maximum excretion of this sugar, even though the assimilation limit has been greatly exceeded. Thus he observes, after an intake of 50 grams of cane-sugar, an excretion of 0.1 gram, while after an intake of 150 grams the excretion was only 0.85 gram.

"Alimentary glycosuria occurs in a healthy person only by saturating the organism with soluble carbohydrates. Therefore it is absent after administering starch, as in this case no more sugar will be absorbed than can be metabolized in the body. It is also scanty, or quite absent, if sugar solutions be given on a full instead of on an empty stomach. Naunyn has observed, regarding alimentary glycosuria, that there is excreted in the urine only that sugar which, according to Ginsberg, reaches the general circulation through the thoracic duct, thus avoiding the liver. Still other external influences may come into play, such as altered capacity of the tissues, especially those of the importantly concerned liver. The result of this may be that excessive doses of glucose are stored up as glycogen or fat in a given time. For saccharosuria and lactosuria the relationships are somewhat different. Here it is very obvious that these double sugars, if given in excessive quantities, are not completely split up in the intestine or during their passage through the intestinal wall, but enter the general circulation as such. The organism, like most of the yeasts, cannot decompose these sugars to any extent, so that they leave the body with the molecules unaffected" (Magnus-Levy).

In testing for the pathologic type of alimentary glycosuria, it is customary to give 100 grams of cane-sugar or glucose either in the morning on an empty stomach or 2 hours after a very light breakfast. The urine of healthy

¹ Woodyatt, Sansum and Wilder (Jour. A. M. A., 1915, LXV, 2067) show that the absorption of sugar, following its oral administration, depends on such varied conditions that an accurate estimation of the sugar tolerance may be made best by intravenous injections of sugar. This tolerance in normal adults is 0.8 to 0.9 gram per kilogram of weight per hour. See, also, Blatherwick and Hawk, Jour. Am. Chem. Soc., 1914, XXXVI, 152; Macleod, Jour. Lab. and Clin. Med., 1916, II, 112; Taylor and Hulton, Jour. Biol. Chem., 1916, XXV, 173; Wilder and Sansum, Arch. Int. Med., 1917, XIX, 311; Bailey, Ibid., 1919, XXIII, 453; Lueders, Ibid., XXIV, 432; Hamman, Can. Med. Assoc. Jour., 1919, IX, 961; Rohdenburg, Bernhard and Krehbiel, Jour. A. M. A., 1919, LXXII, 1528. Photas, Bull. Acad. de Méd., 1920, LXXXIII, 284; Mattill, Mayer and Sauer, Am. Jour. Dis. Child., 1920, XIX, 42; Greenthal, Ibid., 1920, XX, 556; Friedenwald and Grove, Am. Jour. Med. Sc., 1920, CLX, 313; O'Hare, Ibid., 366; Kast, Wardell and Myers, Ibid., 877; McLean and de Wesselow, Quart. Jour. Med., 1921, XIV, 103; Graham, Lancet, 1921, I, 1050; Holst, Ugesk. f. Laeger, 1921, LXXXIII, 1072; Spence, Quart. Jour. Med., 1921, XIV, 314; Neuwirth, Jour. Biol. Chem., 1922, LI, 11.

persons should remain free from sugar, while a pathologically lowered limit of tolerance will be observed by a more or less extensive glycosuria beginning in about 1 hour, reaching a maximum within 2 to 4 hours, and lasting about 8 hours. If this glycosuria follows the administration of starchy foods, the condition is probably a pure diabetic one, while a glycosuria following 100 grams of grape-sugar does not necessarily indicate a diabetes. It is probable that cases showing a somewhat low assimilation limit for sugar belong to the type of mild diabetes, although many class them in the general category of "hepatic insufficiency." That the liver is incapable in such conditions of polymerizing the sugar into glycogen cannot be disputed, but the question at issue is, as von Noorden shows, upon what does the insufficiency of the liver depend? I quote from the article in his *Handbuch der Pathologie des Stoffwechsels* as follows: "The cause cannot be overfilling of the glycogen reservoir, by which we explain the alimentary glycosuria of the healthy. In most cases the subjects are in ill health and their previous diet anything but excessive, so that there is little reason to suppose that their glycogen repository was already filled to overflowing. There is no more reason for supposing that they have a diminished power of utilizing sugar.¹ In none of the affections in question are the processes of oxidation and of energy production decreased; rather is there a great increase in oxidation and especially in the combustion of carbohydrate in certain of the diseases, such as Graves' disease and high fever, which predispose to alimentary glycosuria. Either the liver cells are unable to polymerize all the sugar reaching them or else the glycogen formed is too quickly converted into sugar, through some increase in the diastatic process. The latter would imply that the automatic regulation between sugar combustion and sugar formation is no longer so evenly balanced as in health. Physiologically the diastatic process is dependent only on the sugar requirements of the body; here it would also be controlled by the sugar supply."

The idea of hepatic insufficiency as a distinct clinical entity responsible for the alimentary glycosuria can hardly hold. One should not be satisfied with a mere statement that such a condition exists, but should attempt to explain why it does obtain. In many conditions of the liver in which an undoubted insufficiency is present, no glycosuria can be caused by administration of a fairly large amount of glucose. On the other hand, administration of levulose in these conditions produces a distinct alimentary levulosuria. To the classes of cases which give this levulosuria should be, according to Strauss, applied the term "hepatic insufficiency" rather than to those showing an alimentary glycosuria. (See p. 338.)

It would lead me too far afield to discuss the various factors which influence the appearance of sugar in the urine.² In many cases a restriction of the

¹ Minkowski, Lusk, Murlin and, more recently, Rolly and David assert that this is not true, but that the primary factor in human diabetes is an inadequate utilization of carbohydrates rather than the overproduction of sugar as von Noorden's theory would demand.

² See Barrenscheen, *Biochem. Ztschr.*, 1913, LVIII, 277; Woodyatt, *Jour. Biol. Chem.*, 1913, XIV, 38; Greer, Witzemann and Woodyatt, *Ibid.*, 1913, XVI, 455; Ringer, *Ibid.*, 1914, XVII, 107; Rolly and David, *Münch. med. Wchnschr.*, 1914, LXI, 169; Macleod, *Jour. Am. Med. Assn.*, 1914, LXII, 1222; Sansum and Woodyatt *Jour. Biol. Chem.*, 1914, XVII, 521; Greenwald, *Ibid.*, 1914, XVIII, 115; Woodyatt, *Ibid.*, 1915, XX, 129; Moorhouse, *Patter-*

diet within the assimilation limit will rid the urine of sugar, while in others a constant production of sugar within the system occurs. This endogenous production of sugar may again be of several types. Thus we find in nervous conditions, especially in affections in the region of the fourth ventricle, an excretion of sugar which continues only so long as the glycogen of the body is not used up. If no carbohydrates be taken in the food, glycosuria will soon disappear. This condition is due to interference with the normal nervous control of the glycogenic function of the liver. This type of glycosuria, to which the name "*neurohepatogenous glycosuria*" has been applied, is found in a variety of conditions, such as progressive paralysis, multiple sclerosis, cerebral tumors, peripheral neuritis, traumatic neuroses, mania, melancholia, and hysteria. In this type we find the administration of carbohydrates in the food being followed by a glycosuria, because the diastatic processes are continually urged to increased activity, to such an extent that the hepatic artery becomes loaded with sugar. In other words, we have, in this type, an increased rate of saccharification rather than a diminished formation of glycogen.¹

In other cases a continuous glycosuria is observed even after complete exclusion of carbohydrates from the diet. In this type there is a constant formation of carbohydrates from the protein and fat of the food. The liver appears to be capable of storing up glycogen in the usual amount, but the system in general is practically unable to utilize the sugar brought to it. In consequence of this the liver-cells are repeatedly called upon to convert the glycogen reserve into glucose, and as a result the blood becomes laden with sugar. Even under these circumstances the hyperglycemia would not necessarily cause a glycosuria, providing the normal ferments of the blood arising from the internal secretion of the pancreas were present.² This type of glycosuria is in reality typical diabetes mellitus and is so dependent upon such a large number of factors that the writer must refer to other works for its discussion. Recently Pflüger has shown that a very close relationship exists be-

son and Stephenson, *Biochem. Jour.*, 1915, IX, 171; Sakaguchi, *Mitt. a. d. med. Fak. d. kais. Unive., Tokyo*, 1918, XX, 439; Ervin, *Jour. Lab. & Clin. Med.*, 1919, V, 146; Fitz, *Jour. Am. Med. Assoc.*, 1920, LXXV, 1331; Williamson, *Practitioner*, 1920, CV, 233; Geelmuyden, *Norsk. Mag. f. Lægevidensk.*, 1920, LXXXI, 479; Cammidge, Forsyth and Howard, *Lancet*, 1920, II, 393; Krogh and Lindhard, *Biochem. Jour.*, 1920, XIV, 290; Hewitt and Pryde, *Ibid.*, 395; Blau and Nicholson, *Arch. Int. Med.*, 1920, XXVI, 738; Lesser, *Biochem. Ztschr.*, 1920, CIII, 1; Allen and Wishart, *Jour. Biol. Chem.*, 1920, XLII, 415; *Ibid.*, 1920, XLIII, 129; Mendel and Jones, *Ibid.*, 491 and 507; Allen, *Jour. Exp. Med.*, 1920, XXXI, 363, 381, 555, 575, and 587; *Am. Jour. Physiol.*, 1920, LIV, 375, 425, 439, and 451; *Am. Jour. Med. Sc.*, 1920, CLX, 781; *Ibid.*, 1921, CLXI, 16 and 350; Allen and Wishart, *Ibid.*, 165; Higginson, *Brit. Med. Jour.*, 1921, I, 296; Urechia and Josephi, *Ann. de Méd.*, 1921, IX, 94; Castellani and Willmore, *Brit. Med. Jour.*, 1921, II, 286; Harrison, *Ibid.*, 630; Schmiedeberg, *Arch. exp. Path. u. Pharm.*, 1921, XC, 1; Joslin, *Jour. Am. Med. Assoc.*, 1921, LXXVI, 79; Fitz, *Arch. Int. Med.*, 1921, XXVII, 305; Woodyatt, *Ibid.*, 1921, XXVIII, 125; Beeler and Fitz, *Ibid.*, 804; Sherrill, *Jour. Am. Med. Assoc.*, 1921, LXXVII, 1779; Allen *Jour. Metab. research*, 1922, I, 5, 53, 75, 80, 165, 193, and 221; Martin, *Ibid.*, 43; Allen and Wishart, *Ibid.*, 97; Folin and Berglund, *Jour. Biol. Chem.*, 1922, LI, 213; Wilder, Boothby and Beeler, *Ibid.*, 311.

¹ See Novak, Porges and Strisower, *Ztschr. f. klin. Med.*, 1913, LXXVIII, 413; also, Mann, *Ibid.*, 488; Labbe and Bouchage, *Lancet*, 1914, I, 13. It is to be remembered that emotional excitement, which takes the form of pain, fear, rage, or even slight disturbances of mental equilibrium, may lead to a transitory glycosuria. See Hammett, *Jour. A. M. A.*, 1916, LXVI, 1463.

² See Clark, *Jour. Exper. Med.*, 1916, XXIV, 621.

tween the nervous influences of the duodenum and pancreas. His work apparently indicates that what is generally known as pancreatic diabetes is dependent to some extent upon disturbance of the proper correlation between the duodenum and pancreas. A peculiarity in the true diabetic glycosuria is that other types of sugar, especially levulose, appear to be well tolerated by the diabetic individual without leading to a glycosuria. Besides, the administration of the primary hydrolytic products of glucose, as for instance gluconic and saccharic acids, apparently diminishes an already existing glycosuria. This would seem to indicate that the system is primarily unable to bring about the initial cleavage of the glucose molecule. As it has been shown that many diabetic cases are associated with sclerosis of the islands of Langerhans in the pancreas, this organ is usually regarded as the principal seat of the pathologic changes in true diabetes mellitus. On the other hand, many cases which are clinically indistinguishable from those of pancreatic diabetes show no lesion in this organ postmortem nor any characteristic lesion in other organs.¹

Occasionally, cases are seen in which a diplomellituria exists. This is the contemporaneous or alternate occurrence of diabetic and non-diabetic glycosuria in the same individual (Stern²). The clearing up of one type usually reveals the other. A transient glycosuria may obtain in conditions in which the oxygen supply is reduced, as in suffocation, poisoning with carbon-monoxid, curare and amyl nitrite, or after administration of such drugs as strychnin, cocain, caffein and adrenalin.³ Further, a transient glycosuria is not infrequently seen following the use of alcohol, especially in the form of beer or champagne. A post-anesthetic glycosuria is observed after the use of chloroform and ether, probably as a result of the action of these agents in stimulating the transformation of glycogen into dextrose (Hawk).⁴

Qualitative Tests for Glucose.

The qualitative tests for sugar depend for the most part upon the chemical structure of its molecule.⁵ The hexoses belong either to the class of aldehyds

¹ See Homans, *Jour. Med. Research*, 1914, XXX, 49; Major, *Ibid.*, 1914, XXXI, 313; Bensley, *New York, Med. Jour.* 1915, CI, 523; Kirk, *Arch. Int. Med.*, 1915, XV, 39. For various phases of carbohydrate metabolism see the following: Janney and Blatherwick, *Jour. Biol. Chem.*, 1915, XXIII, 77; Geyelin and Du Bois, *Jour. A. M. A.*, 1916, LXVI, 1532; Epstein and Baehr, *Jour. Biol. Chem.*, 1916, XXIV, 1; Sansum and Woodyatt, *Ibid.*, 327 and 343; Murlin and Sweet, *Ibid.*, 1916, XXVIII, 261; Janney, *Arch. Int. Med.*, 1916, XVIII, 584; Barringer, *Am. Jour. Med. Sc.*, 1916, CLI, 181; Lusk, *Ibid.*, 1917, CLIII, 40; Janney, *Ibid.*, 44; Allen, *Ibid.*, 313; Mackenzie, *Arch. Int. Med.*, 1917, XIX, 593; Fitz, *Ibid.*, XX, 809; McDanell and Underhill, *Jour. Biol. Chem.*, 1917, XXIX, 227, 233, 245, 251, 265, and 273; Palmer, *Ibid.*, XXX, 79; Hoagland and Mansfield, *Ibid.*, XXXI, 501; Benedict, Osterberg and Neuwirth, *Ibid.*, 1918, XXXIV, 217; Kamimura, *Endocrinology*, 1918, II, 330; Kleiner, *Jour. Biol. Chem.*, 1919, XL, 153.

² *Arch. Diagnosis*, 1910, III, 236.

³ See Emerson (*Jour. Am. Med. Assn.*, 1912, LIX, 2245) for a discussion of glycosuria in the insane; also, Herbert, *Biochem. Ztschr.*, 1913, XLVIII, 120; Landau, *Ztschr. f. klin. Med.*, 1913, LXXIX, 201; Folin, Denis and Smillie, *Jour. Biol. Chem.*, 1914, XVII, 519; Luzzatto, *Ztschr. f. exp. Path. u. Ther.*, 1914, XVI, 18; Loewy and Rosenberg, *Biochem. Ztschr.*, 1914, LXI, 180; Eustis, *Am. Jour. Med. Sc.*, 1914, CXLVII, 830; Anders and Jameson, *Ibid.*, 1914, CXLVIII, 323; Pozzo, *Policlinico*, 1915, XXII, 417.

⁴ *Arch. Int. Med.*, 1911, VIII, 30. See also, King, Moyle and Haupt, *Jour. Exper. Med.*, 1912, XVI, 178; Greenwald, *Jour. Biol. Chem.*, 1913, XVI, 375; Ross and Hawk, *Arch. Int. Med.*, 1914, X779, IV.

⁵ See Levene, *Jour. Biol. Chem.*, 1916, XXIV, 59; Hudson and Dale, *Jour. Am. Chem. Soc.*, 1917, XXXIX, 320.

or ketones, and as such will reduce metallic oxids to lower forms. The CHO and CO groups of the aldoses and ketoses, respectively, are the reacting points in all of the reduction tests, such as those with copper and bismuth solutions, as well as in the tests showing the formation of the characteristic osazones. Moreover, these carbohydrates show the peculiarity of fermenting, in the presence of yeast, into alcohol, carbonic acid, and other products. This fermentation test, especially with the *saccharomyces cerevisiæ*, is given only by the sugars having three or a multiple of three carbon atoms in the molecule. Fischer's work has shown that only those sugars may be fermented by a specific ferment in which the ferment and sugar stand to one another in such a relation that a chemical union is possible between them or as he expresses it, only when the ferment fits into the sugar molecule like a key in a lock. This explains why only certain types of the hexoses will ferment in the presence of yeast.

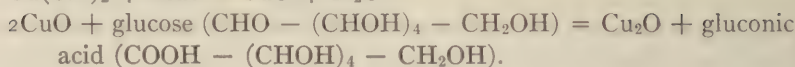
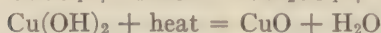
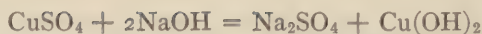
Before any qualitative or quantitative test may be made for the presence of sugar in the urine, albumin must be removed, especially if present in more than traces.¹

Trommer's Test.

To a few c.c. of urine in a test-tube are added one-third its volume of a 10 per cent. solution of sodium hydrate and then, drop by drop, a 10 per cent. solution of copper sulphate. This copper sulphate should be added with constant shaking until a slight excess of the precipitated cupric hydrate ($\text{Cu}(\text{OH})_2$) remains undissolved and is visible, on shaking the tube, as a distinctly greenish-blue flocculent precipitate. The upper layer of the urine is then warmed, when a yellow or red precipitate appears in the heated urine if sugar be present. As a rule, it is unnecessary and even unwise to boil the solution, as otherwise substances other than sugar may produce the reaction. It is true that the reaction is not as sensitive unless the solution be heated to boiling, but other substances do not so readily interfere with the reaction at a lower temperature. The yellow or red precipitate will gradually form throughout the mixture, from above downward, and will finally settle out, leaving a colorless or yellow fluid above.

This reaction is due to the reduction of the cupric hydrate, which is formed by the action of the sodium hydrate upon the copper sulphate, into cuprous hydrate, which becomes dehydrated, on heating, into cuprous oxid. If no sugar be present in the urine and other reducing substances are not excessive in amount, the cupric hydrate will settle out on warming as a black precipitate of cupric oxid. The equations showing these points are as follows:

¹ See Meinertz, *Med. Klin.*, 1913, IX, 1771; Glaserfeld, *Med. Klin.*, 1914, X, 1388, 1413 and 1452; Tirard, *Lancet*, 1914, II, 1133; Geelmuyden, *Norsk Mag. f. Laegevidensk.*, 1915, LXXVI, 985; Folin (*Jour. Biol. Chem.*, 1915, XXII, 327) and Benedict and Osterberg, (*Ibid.*, 1918, XXXIV, 195) have introduced tests which remove practically all the reducing substances in urine with exception of sugar, and thus afford opportunity for detecting the normal urinary sugar. These tests are, however, too delicate for clinical purposes as they show sugar in almost every urine examined. Hiller, *Ibid.*, 1917, XXX, 125, uses a modification of Folin's method to determine pathological glycosurias. See Benedict and Osterberg, *Jour. Biol. Chem.*, 1921, XLVIII, 51; Folin and Berglund, *Ibid.*, 1922, LI, 290.



Whether a yellow or a red precipitate forms will depend upon the alkalinity of the solution, the stronger the alkalinity the more pronounced is the red color due to cuprous oxid, while in less strong alkaline solutions the yellow color of cuprous hydrate will predominate. Certain substances normally present in the urine as well as some which may be added to it have the property of holding in solution the cupric hydrate first formed. This property is shown by the deep blue color which the solution assumes. This color is very intense in the presence of sugar, but it is unwise to assume the presence of sugar from this fact alone. Among the substances which dissolve cupric hydrate and which may be present in the urine in varying amounts we find ammonium compounds, albumin, uric acid, creatinin, allantoin, mucin, glucose, lactose, maltose, pyrocatechin, hydroquinon, alkapton acids, bile pigments, and glycuronic acid. On warming the solution, which may not contain sufficient amounts of glucose to give a typical reaction, a slight reduction of the copper solution will occur leading to the formation of a dirty yellow solution. If these bodies be present in excess a distinct precipitate may occur, and as a result marked confusion may arise regarding the presence of sugar.¹ It has been shown that uric acid and creatinin do not readily reduce at as low a temperature as does sugar, so that slight warming is much better than boiling. The presence of these bodies very frequently leads to a change in color from the bright blue to the greenish-yellow which may be due to the presence of sugar in small amounts, but should never be regarded as indicative of a pathological glycosuria.

It should be a working rule, therefore, that mere decolorization of the fluid should not be regarded as due to sugar. Glucose reduces so much more markedly than the other bodies mentioned that a distinct granular precipitate either of cuprous oxid or hydrate forms and settles out, leaving a supernatant fluid partially or completely decolorized. Normal urine or urine containing an excess of uric acid, creatinin, or ammonium compounds practically never produces an immediate precipitate unless the solution be boiled for some time. This is due to the fact that these substances hold in solution the amount of cuprous oxid which is formed by their reducing action. If such solutions be allowed to stand for a time a reddish-yellow precipitate may occur, but this should not confuse as the typical sugar reaction occurs im-

¹ See Schulz, *Ztschr. f. physiol. Chem.*, 1912, LXXVII, 121; also, Salkowski, *Ibid.*, 1912, LXXIX, 164. According to the more modern theory of Colloid Chemistry, the reducing action, as indicated by the variation in color of the precipitate or the formation of a greenish solution, is due to the formation of cuprous oxid of different degrees of subdivision, that is, possessed of different degrees of dispersion. Substances tending to hold this cuprous oxid in solution, that is, in a state of high dispersion, do not yield the precipitates as readily as they form the greenish solutions. See Fischer and Hooker, *Jour. Lab. and Clin. Med.*, 1918, III, 308; de Jager, *Nederl. Tijdschr. v. Geneesk.*, 1921, I, 2006; Quisumbing and Thomas, *Jour. Am. Chem. Soc.*, 1921, XLIII, 1503.

mediately on warming unless too much copper solution be added. In case only traces of sugar be present, we may obtain little or no positive reaction because the sugar holds in solution the traces of cuprous oxid formed just as do the other substances above mentioned. As a rule, however, sugar in pathologic amounts shows a reducing action over and beyond its dissolving action, so that a red or yellow precipitate must settle out. The limit of this test for sugar is about 0.2 per cent., in which case the reduction will occur as in normal urine without the separation of the characteristic copper precipitate. Even in this case the yellow color is somewhat more intense and is clearer than the dirty yellow color produced in the presence of excessive uric acid or creatinin.

If the urine contain an excess of the conjugated glycuronates, the test may be quite as distinctive as that for sugar. To differentiate such reactions, one should resort either to the phenylhydrazin or the fermentation tests.

Benedict's Test.

This qualitative test¹ is one of the most reliable and accurate modifications of Trommer's test. The reducing action of glucose in alkaline solution is diminished by strong alkalies such as the hydrates. This property may prevent such well-known solutions as Fehling's and Haines' (discussed below) from demonstrating small quantities of sugar. Further, the urine contains many compounds which interfere with the detection of glucose by the strongly alkaline solutions, the loss of delicacy in the other tests being probably due to the fact that normal reduction of the solution is inhibited for a period long enough to allow the strong alkali to decompose the reducing substance. However, in solutions of the alkali carbonates the reduction develops more slowly and is not destroyed by this weak alkali. The addition of sodium citrate, instead of the Rochelle salt as used by Fehling, makes the solution very stable, although little more than does the glycerin of Haines' solution. For the reasons given, this solution of Benedict is more sensitive (about ten times) to urinary sugar than are the other copper solutions. Moreover, it is not appreciably reduced by creatinin and uric acid. It is, however, promptly reduced by the alkapton acids and the conjugated glycuronic as well as by an excess of such preservatives as chloroform, chloral and formaldehyd. The formula of the reagent is as follows:

Copper sulphate (C. P. crystallized),	17.3 grams
Sodium or potassium citrate,	173.0 grams
Sodium carbonate (crystallized), ²	200.0 grams
Distilled water, q. s. ad.,	1000.0 c.c.

¹ Jour. Am. Med. Assn., 1911, LVII, 1193. See, also, Kleiner, *Ibid.*, 1914, LXII, 1307; Sheaff, *Ibid.*, 1915, LXV, 1181; Weinberger, *Am. Jour. Med. Sc.*, 1914, CXLVII, 407; Folin and McElroy (*Jour. Biol. Chem.*, 1918, XXXIII, 513) have introduced a qualitative test for sugar in urine, which has the following composition: 100 grams of sodium pyrophosphate, 30 grams of crystallized disodium phosphate and 50 grams of anhydrous sodium carbonate are dissolved in about 1 liter of water. To this solution is added 13 grams of copper sulphate dissolved in about 200 c.c. of water. The test is used in the same way as is Benedict's but a point of distinction is that, unless a very marked turbidity is noted in the hot solution, the result must be regarded as negative.

² Or one-half the amount of the anhydrous salt may be used.

Dissolve the citrate and carbonate (with aid of heat) in about 700 c.c. of water and filter if necessary. Dissolve the CuSO_4 in about 100 c.c. of water and pour into the alkaline solution. Cool and make up to 1 liter.

The technic is as follows: 5 c.c. of the reagent are placed in a test-tube and not more than 8 or 10 drops of urine are added. The mixture is then heated to vigorous boiling for one to two minutes and allowed to cool spontaneously. In the presence of glucose, the entire body of the solution will be filled with a precipitate which may be red, yellow or greenish in tinge. If the quantity of glucose be low (under 0.3 per cent.) the precipitate forms only on cooling. If no sugar is present, the solution either remains perfectly clear or shows a faint turbidity, which is blue in color and consists of precipitated urates. This test may be especially recommended for all qualitative work.

Fehling's Test.

This is, perhaps, the best-known test for sugar but it is inconvenient and frequently inaccurate for the reasons above mentioned. The formulæ for the solutions are as follows:

Solution A.		Solution B.	
Copper sulphate,	34.64 gm.	Rochelle salt,	173 gm.
Distilled water, q. s., ad.,	500 c.c.	Sodium hydrate	50 gm.
		Distilled water, q. s., ad.,	500 c.c.

In performing the test with Fehling's solution equal parts of solutions A and B are taken and the mixture brought to a boil. The urine is then added, drop by drop, when a reduction of the copper solution will appear in the presence of sugar.¹ The amount of urine added should rarely exceed 10 drops, at the outside 20, if a reduction is to be taken as typical and the solution should not be boiled for more than a few seconds after adding the urine. The addition of larger volumes of urine will usually introduce errors from the factors mentioned under Trommer's test. This test has the same points of interest as has Trommer's test, so that it is wise to dilute the urine before making the test. It is more frequently used than is Trommer's test, but it is not as convenient.

Haines' Test.

Haines has introduced a modification of Trommer's test by adding glycerin, instead of Rochelle salt, to increase the amount of copper in solution. This test is much more convenient than Fehling's, the solution having the advantage of keeping almost indefinitely. It is, however, far less delicate than is Benedict's and is reduced more readily by preservatives as well as by excess of many normal urinary constituents, especially by uric acid and creatinin.²

¹ See Cramer, *Biochem. Jour.*, 1915, IX, 71; Matriri, *Rif. Med.*, 1916, XXXII, 1264; Angiolani, *Policlinico*, 1917, XXIV, 173.

² The creatinin may be almost entirely removed from the urine, if desired, by adding to 10 c.c. of urine about 2 grams of picric acid and about 2 grams of Merck's blood charcoal, shaking for a few minutes and filtering (see Folin, *Jour. Biol. Chem.*, 1915, XXII, 327). See, also, Benedict and Osterberg, *Jour. Biol. Chem.*, 1918, XXXIV, 195.

The composition of Haines' qualitative solution is as follows:

Copper sulphate,	12 grams
Potassium hydrate, ¹	45 grams
Glycerin,	90 c.c.
Water, q. s., ad.,	1000 c.c.

A perfectly clear, transparent, dark-blue liquid results which throws down a very slight reddish deposit of cuprous oxid on standing a week or more.² This does not affect the value of the solution, as the clear blue solution is simply decanted as required.

Four or five c.c. of this solution are placed in a test-tube and gently boiled. Six drops of the suspected urine are added and the upper portion of the mixture brought to a boil and immediately removed from the flame. If sugar be present an abundant yellow or yellowish-red precipitate is thrown down; if no such precipitate occurs sugar is absent. The precautions to be observed in using this test are never to add at the outside more than 10 drops of urine and not to boil the mixture for more than one or two seconds after the addition of urine.

Haines' Modified Test.

Haines and Pond,³ with whom the author collaborated in regard to certain features of the work, have made certain modifications in the above Haines' solution, in order to increase its delicacy and, also, to permit of the performance of the sugar test by the ring or contact method. This modification enables one to detect with certainty 0.03 per cent. of sugar, which is about the average of the so-called "normal" sugar of the urine. In other words, the delicacy of the test is increased so that sugar in pathological amount will be detected. While this test is not as delicate as the special solutions of Folin and of Benedict and Osterberg mentioned on page 319, yet the new Haines' solution has the advantage over these latter solutions that it will show sugar only in pathological amounts, while the other solutions mentioned will show sugar in practically every specimen examined. It is, therefore, a clinical test. Owing to the increase of the specific gravity of the solution, by the addition of the larger amount of glycerin, the employment of the contact test becomes a matter of the greatest simplicity. However, one precaution must be taken before this test may be applied. The phosphates of the urine must be removed before a clear-cut reaction is obtained, as their presence causes the formation of a white ring at the point of contact of the two solutions, which prevents a distinct sugar reaction from showing. This removal may be made by adding to the urine in a test-tube 5 or 6 drops of a 10 per cent. solution of sodium hydrate and allowing the phosphates to settle out or centrifuging if desired to hasten the process. It was thought that this precipitation of phosphates could be averted by the

¹ Or 32 grams of sodium hydrate.

² The glycerin obtainable at present may reduce the solution in a few hours. If the solution be allowed to stand in a warm place for 48 hours, the supernatant fluid may be decanted or filtered and will remain clear for almost an indefinite period.

³ Jour. A. M. A., 1920, LXXIV, 301.

addition of citrate or tartrate to the copper solution, but this was found to be ineffective.

The composition of the modified Haines' solution is as follows:

Copper sulphate.....	5 grams
Glycerin.....	250 c.c.
Potassium hydrate ¹	20 grams
Distilled water to.....	1000 c.c.

Dissolve the copper sulphate in a mixture of the glycerin and an equal amount of water, with the aid of gentle heat. The potassium hydrate should be dissolved in about 200 c.c. of water and added to the copper solution with constant stirring, the whole being made up to volume with distilled water.

The above solution may be used in the same manner as directed for the original Haines' solution, but a much more delicate and beautiful reaction is obtained as follows: Heat about 5 c.c. of the copper solution to boiling in a test-tube, remove from the flame, and hold at an angle of 30 to 40°. To this add, by means of a medicine dropper, 10 to 20 drops of the urine, freed from phosphates as above mentioned, in such a manner that a distinct zone of contact is formed between the copper solution and the urine. The tube is then placed in the upright position and the reaction noted. If sugar is present in quantities exceeding 0.1 per cent., a brick-red or yellowish ring will immediately appear at the junction of the two liquids. If the amount of sugar be less than 0.1 per cent., ranging down to 0.03 per cent., the ring will appear in from a few seconds to slightly less than a minute, the smaller quantities showing slower reactions with a tendency to a more yellowish color of the ring. In urines containing no sugar, no ring of any kind will be noted at the zone of contact.

This test has the following advantages: (1) the reaction is concentrated to a single plane, thus increasing its visibility; (2) only one heating is necessary and no long standing before the reaction appears; (3) it will demonstrate pathological sugar in amounts greater than 0.03 per cent.; (4) it gives a clear-cut decisive result in a minimum of time.

Many other modifications of the copper test have been introduced, both for qualitative and quantitative purposes, but they do not have any advantages over those mentioned especially over Benedict's and Haines' tests. These are used daily in the writer's laboratory and have been found very reliable and serviceable. When other tests are used an error may creep into the report which can be corrected only by resort to fermentation and other confirmatory methods.

Almén-Nylander's Test.

This test is a modification of the original Böttger test and is a distinct improvement. The reagent is prepared as follows: Four grams of Rochelle salt are dissolved in 100 c.c. of 10 per cent. sodium hydrate solution with gentle heat, and as much bismuth subnitrate is added as will dissolve (about 2 grams). After the mixture is cooled the undissolved bismuth subnitrate is filtered off and the filtrate kept in a dark bottle, where it will remain permanent for a long period.

¹ Or 14.3 grams of sodium hydrate.

To a few c.c. of urine in a test-tube is added one-tenth of the volume of this reagent and the mixture boiled for a few minutes. If glucose is present, the fluid will darken and a black precipitate of metallic bismuth separate out. This black precipitate must occur while the solution is being warmed and not after it has cooled. If only a small amount of sugar be present the phosphates precipitated by the alkali may be slightly gray in color instead of the usual white. This test is somewhat more delicate than is the copper test, as it will show about $\frac{1}{40}$ per cent. of sugar.

This reagent is not reduced by uric acid, creatinin, pyrocatechin, hydroquinon or homogentisic acid; but it is reduced by the conjugated glycuronic acid, excess of urinary pigment and pentoses. It is particularly necessary in this test that albumin be removed, as bismuth sulphid forms in the presence of albumin, and may precipitate either in the form of a reddish or a distinctly brownish, even black precipitate. The reduction observed after the administration of medicaments, such as rhubarb, senna, antipyrin, camphor, salicylic acid, salol, sulphonal, trional, quinin, eucalyptus, oil of turpentine, and chloral hydrate, is usually a brown rather than a black unless the solution stands for some time. The administration of saccharin usually results in a reduction of this test, while the copper tests are not affected by it. Sulphur bodies (methyl mercaptan) excreted after the patient has eaten asparagus give a distinct precipitate which may be more or less confusing. If the urine be ammoniacal the reaction may not appear in a characteristic way owing to the fact that the free ammonia is evolved and the alkalinity of the solution is reduced by the combination of the sodium ion with the acid radical formerly bound to the ammonium ion.

This test is used by many as a routine test for sugar, as it is practically always negative with normal urine. The reducing action of the glycuronates and pentoses must, however, be remembered.¹

Fermentation Test.

As previously stated, the reduction tests do not absolutely prove the presence of sugar. All that one can say is that a reducing substance is present and if the reaction be typical the probability is that the reducing substance is sugar. The fermentation test is, perhaps, the most certain of all the tests for glucose and depends upon the fact that only those sugars which contain three or a multiple of three carbon atoms are fermentable with yeast.² Not all members of these groups of sugars will ferment with yeast, so that for absolutely scientific purposes fermentation will not differentiate them. Fischer's work along this line should be carefully read by anyone interested in the biologic properties of the various types of sugar. As the hexoses, which occur in the urine are practically limited to glucose and levulose, we are safe in saying that any sugar fermenting with yeast is one or the other of these monosaccharides, which may be differentiated by tests to be outlined later.

¹ See Mende, Münch. med. Wehnschr., 1914, LXI, 1120; Ewe, Am. Jour. Pharm., 1919, XCI, 717; Trossarello, Rif. Med., 1920, XXXVI, 687; Levine (Science, 1920, LII, 391) has introduced a test for sugar employing a 2 per cent. solution of sodium tellurite in 10 per cent. sodium carbonate as the reagent.

² Boros (Med. Klin., 1913, IX, 874) shows that yeast cells may be so numerous in the urine, as voided, as to destroy the sugar present. This is known as masked or occult glycosuria

The test is performed as follows: Ten c.c. of urine are placed in a test-tube and a piece of compressed yeast, which should be perfectly fresh, about the size of a pea is added and the urine gently shaken until the yeast is finely divided. This mixture is then poured into a fermentation tube, which is allowed to stand in the incubator for a few hours. Two control tests, using in one normal urine and yeast and in the other normal urine, yeast, and a trace of dextrose, are then made and placed in the incubator along with the suspected urine. The presence of sugar is indicated by gas (CO_2) in the upper portion of the fermentation tube. The rapidity of formation of this gas depends upon the amount of yeast as well as upon the age of the yeast. The test indicates from 0.1 to 0.05 per cent. of sugar, especially if the urine be sterilized by previous boiling. The compressed yeast as usually purchased develops a certain minimal amount of gas in normal urine, so that the control test is necessary both to show whether "self-fermentation" is excessive and also whether the yeast is at all active in producing CO_2 from glucose when it has been added. It is necessary, moreover, that decomposition of the urine be prevented, either by previously boiling the urine, addition of a trace of sodium fluorid, or tartaric acid.

This test has the great advantage that the other substances, which reduce copper and bismuth solutions, do not ferment. If precautions are observed to add the right amount of yeast, not to shake the yeast and urine violently enough to include much air, and to prevent bacterial decomposition, this test will positively show the presence or absence of glucose or levulose in the urine and no other substances. It is always wise to use this test either as confirmatory or decisive in conjunction with the previous reduction tests.

Phenylhydrazin Test.

This test is much more delicate than any of the previous tests mentioned. Theoretically it will show sugar in the amount present in normal urine, but practically no definite reaction is observed. The principle of the reaction is the decomposition which occurs between the aldehyd or ketone group of the sugar molecule and the amino group of the phenylhydrazin. In this reaction, characteristic bodies known as hydrazones are first formed, which are converted, in the presence of dilute acetic acid and an excess of phenylhydrazin, into crystalline bodies known as osazones. These latter bodies are characteristic of the sugar group, usually differing from one another, depending upon the original sugar from which they were formed. The osazones crystallize in definite forms, the purified crystals showing rather sharp melting points. It is, therefore, essential not only that a crystalline body be obtained in this reaction, but that the crystalline form and the melting-point of the crystal be that characteristic of the sugar suspected. The success of the test will depend largely on the relation of the sugar to the reagents, the best proportions being approximately one of sugar, two of phenylhydrazin, and three of sodium acetate. All of the members of the hexose and pentose groups show this reaction, as do many of those of the polysaccharide series. As will be seen from the reaction given below, those sugars which differ only in the space-relations of the atoms attached to the first two carbon atoms can possibly

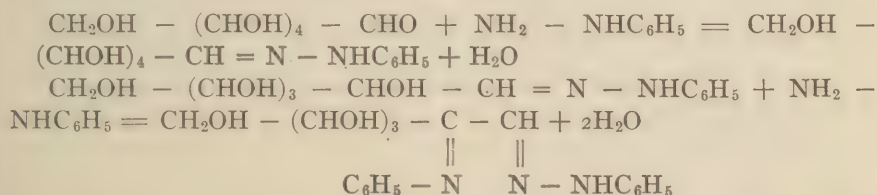
PLATE IX.



OSAZONS. (*Hawk.*)

Upper form, dextrosazon; central form maltosazon; lower form, lactosazon.

give the same osazone. For this reason we find glucose, levulose, and mannose as well as glucosamine giving exactly the same osazone (phenylglucosazone), showing a characteristic yellow needle-shaped crystalline deposit, with a melting-point of 204 to 205°C. The reactions leading to the formation of this body are shown in the following equations:



Twenty-five c.c. of urine are treated with a few drops of a solution of lead acetate and filtered to remove the albumin if present. To the filtrate, which must be acidified with acetic acid if it is not already acid, is then added phenylhydrazin hydrochlorate ($\frac{1}{2}$ to 1 gram) and about 2 grams of sodium acetate. The tube is then shaken thoroughly to mix the contents and is placed in a boiling water-bath for from one to two hours. Some workers recommend a shorter period, such as 20 minutes, but the writer has never been able to get as good results with the short heating. At the end of this time the material in the tube is filtered while hot and the filtrate allowed to cool slowly rather than as some advise to cool suddenly by immersion in cold water. If glucose be present a yellow crystalline deposit will appear, which under the microscope will show the characteristic needle-shaped crystals arranged in bundles or sheaves. This microscopic examination does not absolutely prove the crystals to be phenylglucosazone so that a melting-point determination must be made before definite proof is forthcoming. For this purpose the crystals must be purified by dissolving in a hot 60 per cent. alcohol and recrystallizing by adding water and evaporating the alcohol. A few of these crystals are then placed in a perfectly dry capillary tube and the melting-point determined by methods previously learned in organic chemistry.

Neumann has applied Fischer's method of using this test to the urine. Five c.c. of urine are treated in a test-tube with 2 c.c. of a 50 per cent. solution of acetic acid saturated with sodium acetate and two drops of pure phenylhydrazin. This mixture is then evaporated by boiling to 3 c.c., after which it is cooled quickly and is then rewarmed and allowed to cool slowly. If glucose be present to the amount of 0.02 per cent., pure crystals of phenylglucosazone separate out in 5 to 10 minutes. If the urine has a high specific gravity and a low sugar content the crystals do not form so quickly. This test is much to be preferred in well-equipped laboratories, but is not as convenient as the use of the crystalline phenylhydrazin hydrochlorate for the general worker, as the fluid phenylhydrazin is quite irritating and is not as easy to work with.

This test cannot be used for quantitative purposes as the yield is never complete. For qualitative purposes, however, it is to be especially recom-

mended. When properly applied it is the most delicate and one of the most reliable tests at our disposal. If the melting-point of the crystals be determined the only confusing substances will be levulose, glucosamin, and manose. The osazones formed from the other carbohydrates and the glycuronic acid compounds crystallize in somewhat similar form, but do not show the characteristic melting-point of 204° to 205°C. of the phenylglucosazone. It is true that the impure crystals melt at somewhat lower temperature, but if carefully purified from 60 per cent. alcohol they will melt at approximately 204° .

Quantitative Methods.

Folin and Peck's Method.

This method¹ is a revision of that of Folin and McElroy,² which, in turn, is a modification of that of Benedict.³ It is accurate and reliable, if all precautions noted are followed, and can be recommended.

Reagents Necessary.

1. A copper sulphate solution, containing 59 grams of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 2 c.c. of concentrated sulphuric acid per liter. The acid is added to prevent the gradual decomposition of the copper sulphate to copper hydrate or silicate, which is presumably caused by the solvent action on the glass.

2. An approximately saturated sodium carbonate solution (14 to 20 per cent. Na_2CO_3).

3. A salt mixture containing 200 grams of crystallized disodium phosphate, 50 grams of sodium thiocyanate (or 60 grams of the potassium salt), and 100 grams of anhydrous sodium carbonate (or 120 grams of monohydrated sodium carbonate. As difficulties have been encountered in the proper preparation of this mixture, as pointed out by Haskins⁴ and others, the following method should be followed: Powder in a large mortar 200 grams of the disodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) and sprinkle over it the 50 grams of sodium thiocyanate. Mix for 10 minutes with mortar and spoon giving a uniform semi-liquid paste. Add the sodium carbonate and mix with mortar and spoon until a rather fluffy granular powder is obtained. Test this mixture for proper mixing by adding 5 grams of it to 5 c.c. of the above copper sulphate solution; if any black specks are formed, even temporarily, the mixture is incomplete; a certain amount of green color is, however, practically unavoidable. If no black coloration is obtained, the mixture is satisfactory. Allow the mixture to stand in the mortar over night (covered with paper) and place in well stoppered bottles for future use.

Technic.

Fill a special sugar buret, graduated in 0.02 c.c., by suction with the urine to be tested. These burets should have accessory tips, which are drawn out to almost a capillary point in order to permit the delivery of a large number of drops to the unit of measure. This precaution does

¹ Jour. Biol. Chem., 1919, XXXVIII, 287.

² Ibid., 1918, XXXIII, 513.

³ Ibid., 1911, IX, 57; Jour. A. M. A., 1911, LVII, 1103. See Smith (Jour. Lab. and Clin. Med., 1922, VII, 364) for a minor modification of Benedict's Method.

⁴ Jour. Biol. Chem., 1919, XXXVII, 303.

away with the necessity of dilution of highly saccharine urines before making the determination. Albumin does not interfere with this test, although it does alter the appearance of the precipitate of cuprous thiocyanate which forms, rendering it more flocculent and bulky.

Transfer 5 c.c. of the above copper sulphate solution to a large hard glass test-tube. Add approximately 1 c.c. of the saturated sodium carbonate solution; shake for a moment, and then add 4 to 5 grams of the above salt mixture. Heat gently with shaking until all the salts have been dissolved, except for a few isolated particles of sodium carbonate. A clear solution is usually obtained in less than 1 minute at temperatures which need not exceed 60°C. Now add the urine, 0.4 to 1 c.c., heat fairly rapidly to boiling, and then boil very gently so as not to drive off too much water. When the mixture begins to bump add a small glass bead. If the boiling contents of the test-tube do not suddenly become filled with the white precipitate of cuprous thiocyanate within the first 15 seconds of boiling, then less than $\frac{1}{2}$ of the required sugar has been added and more of the urine must be added without delay. If the full required amount of sugar is present in the urine first added, the solution becomes turbid 5 seconds after the boiling point has been reached. The only time restriction called for in connection with the final titration is that complete reduction, as evidenced by decoloration of the solution and precipitation of the white cuprous thiocyanate, must not occur in less than 4 minutes boiling. The volume of the solution in the test-tube should not become less than 6 to 7 c.c.

Five c.c. of this copper solution are reduced by 25 mg. of dextrose or levulose, 45 mg. of anhydrous maltose, or 40.4 mg. of anhydrous lactose.

Bang's Method.

This method¹ appears to the writer to be one of the best at our disposal for the quantitative estimation of sugar in the urine. Like the solution of Benedict previously mentioned, it contains alkali carbonates instead of hydrates. The principle of this method is as follows: The urine is treated with an excess of standard alkaline copper solution and boiled to bring out the reduction due to sugar. The amount of copper remaining in excess is then determined by titration with a solution of hydroxylamin sulphate (instead of with potassium sulphocyanate as used by Citron) and from this the amount of sugar is calculated.

The solutions necessary in this test are as follows:

(a) One hundred grams of potassium bicarbonate are dissolved in about 1300 c.c. of distilled water contained in a 2-liter flask. To this solution are added 500 grams of potassium carbonate and 400 grams of potassium sulphocyanate. Exactly 25 grams of pure copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) are then dissolved in about 150 c.c. of warm distilled water. After cooling this solution is added quantitatively to the carbonate solution. Add water up to the

¹ Biochem. Ztschr., 1907, II, 271; 1908, XI, 538; 1911, XXXII, 443. Bang (Ibid., 1913, XLIX, 1) has devised a new method which is cheaper and equally exact. See, also, Citron, Münch. Med. Wehnschr., 1918, LXV, 1053.

2-liter mark, allow to stand for 24 hours and filter. This solution is stable for about three months.¹

(b) Two hundred grams of potassium sulphocyanate are dissolved in about 1500 c.c. of distilled water in a 2-liter flask. 6.55 grams of hydroxy-

BANG'S TABLE OF REDUCTION EQUIVALENTS

Cubic centimeters of hydroxylamin solution	Milligrams of sugar	Cubic centimeters of hydroxylamin solution	Milligrams of sugar
0.75	60.0	25.50	23.5
1.00	59.4	26.00	22.9
1.50	58.4	26.50	22.3
2.00	57.3	27.00	21.8
2.50	56.2	27.50	21.2
3.00	55.0	28.00	20.7
3.50	54.3	28.50	20.1
4.00	53.4	29.00	19.6
4.50	52.6	29.50	19.1
5.00	51.6	30.00	18.6
5.50	50.7	30.50	18.0
6.00	49.8	31.00	17.5
6.50	48.9	31.50	17.0
7.00	48.0	32.00	16.5
7.50	47.2	32.50	15.9
8.00	46.3	33.00	15.4
8.50	45.5	33.50	14.9
9.00	44.7	34.00	14.4
9.50	44.0	34.50	13.9
10.00	43.3	35.00	13.4
10.50	42.5	35.50	12.9
11.00	41.8	36.00	12.4
11.50	41.1	36.50	11.9
12.00	40.4	37.00	11.4
12.50	39.7	37.50	10.9
13.00	39.0	38.00	10.4
13.50	38.3	38.50	9.9
14.00	37.7	39.00	9.4
14.50	37.1	39.50	9.0
15.00	36.4	40.00	8.5
15.50	35.8	40.50	8.1
16.00	35.1	41.00	7.6
16.50	34.5	41.50	7.2
17.00	33.9	42.00	6.7
17.50	33.3	42.50	6.3
18.00	32.6	43.00	5.8
18.50	32.0	43.50	5.4
19.00	31.4	44.00	4.9
19.50	30.8	44.50	4.5
20.00	30.2	45.00	4.1
20.50	29.6	45.50	3.7
21.00	29.0	46.00	3.3
21.50	28.3	46.50	2.9
22.00	27.7	47.00	2.5
22.50	27.1	47.50	2.1
23.00	26.5	48.00	1.7
23.50	25.8	48.50	1.3
24.00	25.2	49.00	0.9
24.50	24.6	49.50	0.5
25.00	24.1	50.00	0.0

For every $\frac{1}{10}$ c.c. hydroxylamin solution used more than is given in the table, subtract 0.1 mg. if the reading be between 49 and 15, while if it be between 15 and 1 subtract 0.2 mg.

¹ Hatta (Biochem Ztschr., 1913, LII, 1) shows that the self-reduction of this solution may give as high an error as 9% on the positive side.

lamin sulphate are dissolved in water and added quantitatively to the sulphonyl cyanate solution. Add water up to the 2-liter mark and preserve the mixture in dark colored bottles. This solution is very permanent.

Technic.

Ten c.c. of urine (5 or 2 c.c. diluted with water to 10 c.c. if more than 0.6 per cent. sugar is present) are measured into a 200 c.c. Jena Erlenmeyer flask and treated with 50 c.c. of the copper solution (a). Heat on a wire gauze to boiling for *exactly* three minutes. Cool quickly to room temperature by immersion of the flask in cold water. Now titrate with the hydroxylamin solution (b) until a colorless mixture results. From the number of c.c. of hydroxylamin sulphate solution used, calculate the sugar in milligrams by means of the accompanying table. A simple calculation yields the percentage and total amounts.

It has been found difficult to get a sharp end-point in this reaction if the urine be rather highly colored. Bang and Bohmansson¹ have shown that the urine may be cleared by the following process. Twenty c.c. of urine are treated with 5 c.c. of 25 per cent. HCl and 2 grams of blood charcoal. Shake a few times during five minutes and filter through a dry filter into a dry beaker. This filtrate is then used for the test as described above. Woodyatt and Helmholtz² and Andersen³ have called attention to the necessity of testing the charcoal by control experiments before using the above method of clearing. As some of the blood charcoals give very erroneous results, they recommend the use of Merck's preparation. A further advantage of using this clearing method is that the "auto-reduction" of the urine is reduced to a low limit owing to the almost complete removal of urochrom, uric acid, creatinin and glycuronic acid derivatives.⁴

Purdy's Method.

It has been found that the addition of strong ammonia to the mixed copper and tartrate solutions makes the end-point much more distinct. Pavy, Sahli, Kumagawa and Suto, Kinoshita, and others have modified the original Fehling's solution in this way. It was shown, however, by Löwe that the substitution of glycerin for the Rochelle salt made a much more stable solution and one which could be originally mixed and kept for indefinite lengths of time. Purdy has succeeded in obtaining a copper solution which, in the writer's experience, is much to be preferred for general laboratory purposes over most of the other modifications of Fehling's solution. His formula is as follows:

Chemically pure copper sulphate,	4.752 grams
Potassium hydrate, ⁵	23.500 grams
Strong C. P. ammonia (sp. g. 0.88),	350.000 c.c.
Glycerin,	38.000 c.c.
Distilled water, q. s. ad.,	1,000.000 c.c.

¹ Ztschr. f. physiol. Chem., 1909, LXIII, 443; Biochem. Ztschr., 1909, XIX, 281.

² Arch. Int. Med., 1911, VII, 598.

³ Biochem. Ztschr., 1911, XXXVII, 262. See Hall, Jour. Indus. & Eng. Chem., 1922, XIV, 18.

⁴ Bang (Biochem. Ztschr., 1912, XXXVIII, 168) has recently advised the substitution of 2 c.c. of 95% alcohol for the 5 c.c. of HCl.

⁵ Or 16.8 grams of sodium hydrate.

This solution is prepared by dissolving the copper sulphate and glycerin in 200 c.c. of distilled water, heating gently if necessary. The potassium hydrate is dissolved in a second 200 c.c. of water and mixed with the copper solution. When the mixture has cooled add the ammonia and bring the total volume up to 1 liter with distilled water. Thirty-five c.c. of this solution are decolorized by 0.02 gram of glucose.

Haines has slightly modified this original solution of Purdy so that 10 c.c. of the solution are decolorized by 0.01 gram of glucose. This titer is somewhat more convenient than that of Purdy as the calculation is distinctly simplified. The formula for his modification is as follows:

Pure copper sulphate,	8.314 grams
Pure potassium hydrate, ¹	25.000 grams
Glycerin,	40.000 c.c.
Ammonia,	350.000 c.c.
Distilled water, q. s. ad.,	1,000.000 c.c.

The principle of this test depends upon the fact that, in the reduction of cupric oxid in solutions of definite strength by glucose, the blue coloration disappears on the addition of a definite amount of glucose without any attendant precipitate, the reduced solution remaining transparent and colorless.

Technic.

Thirty-five c.c. of Purdy's test solution or 10 c.c. of Haines' solution are measured into an Erlenmeyer flask and 50 c.c. of water added. The flask is then closed with a doubly perforated rubber stopper, through one hole of which passes the stem of a buret containing the suspected urine and through the other a bent glass tube to conduct the fumes of ammonia away from the observer. The object of closing the flask with the stopper is to exclude the air and thus prevent reoxidation of the cuprous oxid. The contents of the flask are now brought to a gentle boil and the urine added, 1 c.c. at a time, shaking the flask after each addition until the fluid is completely decolorized. The number of c.c. used is then noted and a second test made, adding at once 1 c.c. less of urine than the total number of c.c. used in the first experiment. The last portions of the urine are added two drops at a time, allowing from three to five seconds to elapse between the addition of these separate portions. When the urine is completely decolorized the number of c.c. used is noted. As the amount of urine used is equivalent to 0.02 gram of glucose with Purdy's solution or 0.01 gram with Haines' solution, the percentage may be obtained as in the previous method. It is necessary with this method that the urine be diluted for the same reasons as previously mentioned.

In all of the copper tests for glucose the influence of preservative agents must be remembered. Thus chloroform, chloral hydrate, and formalin will all reduce copper solutions so that an error may be introduced unless these substances are removed.

In selecting a method for the quantitative determination of sugar in the urine, the general worker should remember that the method used should be both simple and exact. With these points in view, the writer has little hesitancy in recommending Folin and Peck's or Bang's method. If the urine be cleared

¹ Or 18 grams of sodium hydrate.

as previously described, the results are all that could be desired, the error being extremely small. Many other modifications of the copper tests have been advocated, but the writer does not feel that they have any advantage from either the scientific or clinical standpoint. The method of Allihn, in which a copper solution is reduced and the precipitated cuprous oxid either weighed as such or further reduced to metallic copper in a stream of hydrogen, is not clinically available. The modifications of Rudisch, Rudisch and Celler, and of Gerrard and Allan do not give as accurate results as do the above methods in the experience of the writer.¹

Polariscopic Method.

In this test the urine must be absolutely clear and must contain no albumin. Moreover, it is essential that such substances as glycuronic acid and β -oxybutyric acid be removed before polarization as they will introduce con-

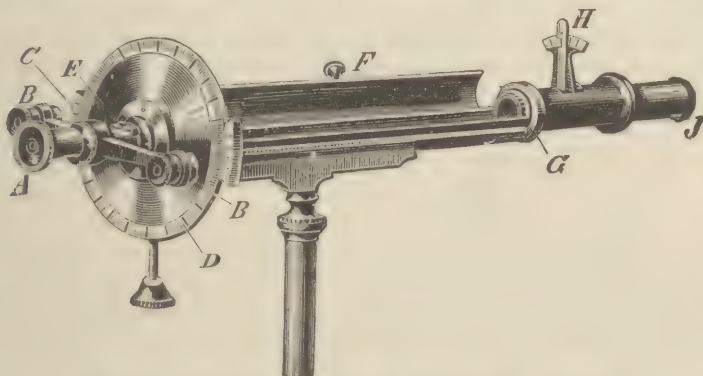


FIG. 84.—One form of Laurent Polariscope. (Hawk.)

B, Microscope for reading the scale; C, a vernier; E, position of the analyzing Nicol prism; H, polarizing Nicol prism in the tube below this point.

siderable error in the reading. The best clearing agent is that, previously mentioned under Bang's test, of shaking the urine with HCl and blood charcoal and filtering. Instead of this method the urine, acidified with acetic acid, may be treated with a solution of normal lead acetate, which will precipitate the albumin and remove excess of pigment. The mixture is filtered and the clear filtrate is used for the test. It must be remembered that a correction must be made in the percentage of sugar in the filtrate, if the urine be cleared with a solution of lead acetate. This may be done by adding 25 c.c. of a 10 per cent. solution of lead acetate to 75 c.c. of urine. The sugar in the filtrate will represent only three-fourths of that of the original urine. Basic lead acetate should be used with caution, as it precipitates various sugars. If the urine contain no albumin, magnesium oxid or silicic acid may be used as clearing agents.

Various types of polarimeters have been suggested and most of them

¹ See Nagasaki, *Ztschr. f. physiol. chem.*, 1916, XCV, 61; Wilson and Atkins, *Biochem. Jour.*, 1916, X, 504; Vansteenbergh and Bauzil, *Paris Méd.*, 1916, VI, 556; 1917, VII, 164. Cammidge, *Lancet*, 1917, I, 613; *Ibid.*, 1919, I, 939; Utz (*Süddeutsch. Apoth. Ztg.*, 1919, LIX, 280) uses methylene blue as his reagent; Goiffon and Nepveux, *C. R. soc. de biol.*, 1920, LXXXIII, 121; Ionescu and Vargolici, *Bull. Soc. Chim. Romania*, 1920, II, 38; de Saint-Ray and Ronfaut, *Bull. soc. pharmacol.*, 1920, XXVII, 289; Judd, *Biochem. Jour.*, 1920, XIV, 255; Baker and Hulton, *Ibid.*, 754; Shaffer and Hartmann, *Jour. Biol. Chem.*, 1921, XLV, 349 and 365; Sumner (*Ibid.*, 1921, XLVII, 5) employs an alkaline solution of dinitrosalicylic acid as his reagent.

are quite satisfactory for the determination of sugar. One of these with its description is seen in the accompanying cut and legend. The principle upon which their use depends is the fact that optically active substances when in solution have the power of turning the plane of polarized light either to the right or the left. The zero point of the instrument is determined by observing the point at which the halves of the optical field have exactly the same degree of illumination, when the light passes through a tube either empty or containing an optically negative fluid. The point at which the graduated scale of the instrument and the vernier correspond is regarded as the zero-point. In the use of the polariscope any deviation of the plane of light passing through the polarizing Nicol prism will be noticed by a darkening of one portion of the field, so that the compensating or analyzing Nicol must be rotated until both parts of the field are equally illuminated. In this way one readily determines how much the plane of polarized light has been deviated, by observing the degree of rotation necessary to bring the two portions of the field into equal illumination when the light passes through an

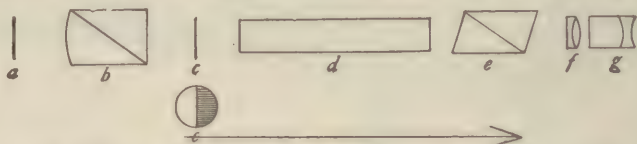


FIG. 85.—Diagrammatic representation of the course of light through the Laurent polariscope. (Direction reversed from that of previous figure.) (*Hawk.*)
a, Bichromate plate to purify the light; *b*, the polarizing Nicol prism; *c*, a thin quartz plate covering one-half the field and essential in producing a second polarized plane; *d*, tube to contain the liquid under examination; *e*, the analyzing Nicol prism; *f* and *g*, ocular lenses.

optically active fluid. The most reliable instruments for general work are known as the "half-shadow" types, but they rarely find a place in the equipment of the general worker. The examination is usually made in a dark room, the light passing through the tube of the polariscope from a sodium flame.

It must be remembered that the urine may contain substances other than glucose which rotate the plane of polarized light. The normal urine is slightly levorotatory, the degree varying between 0.05 and 0.18. Albumin is also levorotatory and will interfere markedly with the degree of rotation referable to glucose, unless it be very slight in amount or be entirely removed. Levulose, β -oxybutyric acid, and the conjugated glycuronates are also levorotatory, the second being especially prone to interfere with the glucose rotation as it so frequently is associated with glucose in diabetic conditions. If the urine has been previously heated with acid before being examined in the polariscope, glycuronic acid will also interfere, as it shows distinct dextrorotation. Cane-sugar as well as lactose are occasionally found in the urine and may lead to confusing results as both are dextrorotatory substances. It is, therefore, necessary before any reliable results may be obtained with the polariscope to remove the interfering substances, either as preliminary to the determination of glucose or subsequently, a correction being then made for the rotation of the interfering substance. It is to be said that the polariscope is more fitted for a clinical laboratory than for the general practitioner.

The rotation of light when passing through optically active solutions is dependent upon several factors, among which we find the temperature at which the observation is made, the length of tube through which the light passes and the concentration of the active solution. By specific rotation of a fluid is meant the rotation observed when light passes through a solution containing 1 gram of the active substance per c.c. of fluid and placed in a tube one dcm. in length. Thus we find the specific rotation of glucose in such a tube being $(\alpha)_D = +52.74$. It is, therefore, evident that the percentage of sugar in an unknown solution contained in a tube 1 dcm. in length may be obtained by dividing the degree of rotation by 0.5274. Some of the tubes for clinical purpose are constructed of such a length (188.6 mm.) that one degree of rotation equals 1 per cent. of glucose. For a full discussion of the subject of optical activity of fluids the writer would refer to the work of Landolt.¹

Technic.

Having determined the zero-point of the instrument, the tube, which should be thoroughly cleaned and dry, is filled with the fluid to be examined. In filling this tube precautions must be taken not to include any bubbles of air, which is best done by filling the tube until a convex meniscus is observed and then sliding the glass disk over the end in such a way that the excess of fluid is shoved off. The metal cap is then screwed on tightly, but in such a way that undue compression is not exerted upon the glass disk. The tube is placed in position, the field distinctly focussed and the degree of rotation determined by revolving the analyzing Nicol until the two portions of the optical field have exactly the same intensity of illumination. This will require considerable experience, as the accuracy of the determination will depend not only upon the clearness of the fluid, the degree of focussing, the sensitiveness of the instrument, and the brightness of the light, but also upon the sensitiveness of the observer's eyes. Several readings should be made, turning the prisms from both directions and observing the degree at which the fields are isochromatic, the average being taken as the final result. As the eyes soon become fatigued, they should be used for only a few seconds at a time.

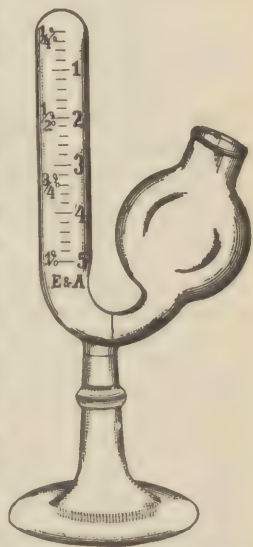


FIG. 86.—Einhorn saccharometer.

Having determined the degree of rotation, the percentage of sugar is then calculated as previously mentioned. This method, if carefully used and interfering substances avoided, is the most accurate method for the determination of glucose. It is, however, difficult, requires much experience, and is not as sensitive as the other methods, rarely detecting the presence of less than 0.2 per cent. of glucose. Theoretically, the percentage of sugar as determined by the polariscope should exactly agree with that obtained by titra-

¹ Das optische Drehungs-Vermögen, Braunschweig, 1898.

tion of copper solutions, but in general work such is never the case, as interfering substances may be present or have not been completely removed.

The writer must refer elsewhere for methods of correcting the readings of the polariscope when interfering substances are present, as he does not think it wise to recommend the general worker to waste his time with this instrument when sufficiently accurate clinical results may be obtained by methods which are for him much easier and less liable to error.

Fermentation Methods.

The principle of this method has been previously outlined. It has been found that, if the precautions mentioned are observed, carbon dioxide is evolved quantitatively from glucose by the action of yeast. The most convenient method of applying the test is to use an Einhorn *fermentation tube* which is so graduated that the amount of CO_2 evolved is directly read off in terms of per cent. of glucose. For this purpose the urine must contain less than 1 per cent. of glucose, the urine being diluted as previously described to bring the amount within this figure, multiplying the result, of course, by the degree of dilution. The urine, which should be acid in reaction, is shaken with a piece of compressed yeast about the size of a small pea, all precautions being observed as mentioned above. The mixture is then poured into the fermentation tube in such a way that no air-bubbles collect at the upper end of the tube. Controls as previously described are then made and the three tubes placed in the incubator at 37°C . In a few hours bubbles of gas (CO_2) will collect at the top of the tube, the fermentation being practically complete overnight. The percentage of sugar is then read direct from the calibration ($1/4-1$ per cent.) on the tube (the figures from one to five representing c.c. of gas and not per cent. of sugar).

Lohnstein's Saccharometer.

This apparatus is seen in the accompanying cut. Twelve c.c. of mercury are placed in the bulb of the apparatus. One-half c.c. of the urine to be tested is then floated upon the mercury and treated with a thick paste of compressed yeast diluted two or three times with water. The stopper is then carefully greased with vaseline and inserted so that the two apertures correspond. By tipping the apparatus a trifle the column of mercury in the long tube is then adjusted to the zero point of the scale. When this is done the stopper is turned so that the holes no longer correspond and the weight is placed on the stopper to prevent leakage from the increased pressure of the gas liberated in the fermentation. The apparatus is then placed in the incubator and the extent of fermentation read off by noticing the height to which the column of mercury rises in the long arm of the instrument. The fermentation is usually complete within six hours. After removing the apparatus from the incubator it is allowed to stand in the air for a few minutes to adjust itself to room temperature, as the scale is graduated in this way.

This method gives results which correspond very closely to those of titration and is to be recommended for the quantitative determination by fermentation methods.

Lohnstein has also introduced a saccharometer which may be used with

dilute urine. It is seen in the accompanying cut. It does not, in the writer's opinion, have any advantage over the above-mentioned apparatus as the principle is the same, although the urine must be diluted.

Robert's Method.

This method has been recommended for the quantitative determination of sugar and is based upon the fact that the specific gravity of the urine is changed in a quantitative way when the sugar of the urine is fermented.



FIG. 87.—Lohnstein's fermentation tube for undiluted urine.

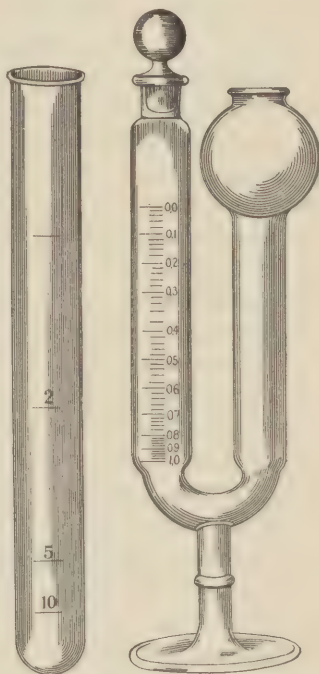


FIG. 88.—Lohnstein's fermentation tube for diluted urine.

The urine must be acid before applying this test. A piece of yeast about the size of a bean is added to the urine which is allowed to ferment at incubator temperature until no further qualitative test for sugar is obtained. This will usually require from 24 to 48 hours, so that a trace of sodium fluorid should be added to the urine to prevent bacterial action. The specific gravity of the urine before it is subjected to fermentation is very carefully taken either with a very accurately standardized hydrometer or, preferably, with the pycnometer. After fermentation is complete the specific gravity of the fermented urine is determined in the same way. The difference in the specific gravity of the two specimens is then multiplied by 234 to obtain the percentage of sugar. Or, according to Purdy, each degree of specific gravity lost in fermentation corresponds to 1 grain of sugar per fluidounce.

If the specific gravity be accurately determined the results are correct

within 0.1 per cent. As this method requires, however, the use of a pycnometer and a very accurate chemical balance, it can hardly be recommended to the general practitioner for his work.

(b) **Levulose (d-fructose), $\text{CH}_2\text{OH}-(\text{CHOH})_3-\text{CO}-\text{CH}_2\text{OH}$.**

Levulose is found very widely distributed throughout the vegetable kingdom, especially in fruits. Honey is almost a pure levulose. It may be found in the urine, transudates, or exudates, after a large intake of levulose-containing food or may occur spontaneously, when the subject has taken little such food. The *levulosuria*, reported by Zimmer, Ventzke, Czapek, Worm-Müller, Seegen, Mauthner, Cotton, Roehmann, Personne, Henninger, Marie, and Robinson, must be accepted with reserve, as the incompleteness of the methods then in use afforded no certain means of recognizing levulose (Neuberg¹). The most authentic cases of true levulosuria are those of May, Schlesinger, Rosin and Laband, Lépine and Boulud, and Neubauer. This pure levulosuria occurs in both sexes and at all ages, the amount of sugar excreted being subject to variations from 2.7 grams per diem (Schlesinger) to 24 grams (Lépine).

More common than pure levulosuria is its association with a glycosuria. This combination appears in all forms of diabetes, in the severe types levulose being practically never missed, according to Umber, especially when no restriction is placed on the carbohydrate intake. Neubauer's observations on this point are interesting. He finds that withdrawal of carbohydrates causes both levulose and dextrose to disappear from the urine. When levulose was given it was utilized, but when glucose was taken it was less completely assimilated, being excreted in part as levulose.

The tolerance for levulose is, as a rule, less than that for glucose, so that we are not surprised to find that the administration of 100 grams of levulose to normal individuals is followed by a levulosuria in about 10 per cent. of cases, while no such effect may be observed in diabetes mellitus. Here the question of individual tolerance must be considered, as Umber finds 25 grams of levulose excreted following an intake of 100 grams, while the writer has observed an excretion of 75 grams on the same intake. Strauss² finds that an alimentary levulosuria occurs, after an intake of 100 grams of levulose, in 90 per cent. of cases of functional hepatic disturbance. This test would seem, therefore, to be a valuable indication of hepatic insufficiency, although it is not pathognomonic. Also *alimentary galactosuria*, following the administration of 40 grams of galactose to the fasting stomach, as originated by Bauer,³ has been shown by Reiss and Jehn and, also by Roubitschek to be inconstant. A decidedly positive excretion of galactose is observed only in cases in which the entire liver parenchyma is presumably involved, as in the acute infections and intoxications characterized by catarrhal jaundice.

¹ Handbuch der Pathologie des Stoffwechsels, Berlin, 1907, 716; also, Cammidge and Howard, Lancet, 1915, I, 320.

² Deutsch. med. Wchnschr., 1901, XXVII, 757 and 786; Ibid., 1913, XXXIX, 1780. See, also Oszacki and Wagner, Med. Klin., 1913, IX, 1549; Draudt, Arch. f. exper. Path. u. Pharmakol., 1913, LXXII, 457; Hohlweg, Münch. med. Wchnschr., 1913, LX, 2271; and Schirokauer, Ztschr. f. klin. Med., 1913, LXXVIII, 462.

³ Deutsch. med. Wchnschr., 1908, XXXIV, 1505.

Cases of cirrhosis, carcinoma, cholelithiasis and phosphorus poisoning react either negatively or very slightly.¹

The chemical reactions of levulose are very similar to those of dextrose. Owing to the presence of the ketone group in its molecule, it shows the same reducing actions as does the aldehyd group of the glucose. Like glucose it ferments, but not quite so readily. It is levorotatory, its specific rotation being $(\alpha)_D = -91$ degrees.² Leo has reported the finding of a levogyrate carbohydrate in diabetic urines, which he believes to be laiase. This differs from levulose in being unfermentable with yeast. Levulose forms exactly the same phenylosazon as does glucose, so that it is a matter of great difficulty to differentiate these bodies by the tests given above. Lobry de Bruin and Alberda von Eckenstein have shown that glucose and levulose may change, the one into the other, by the action of traces of alkali, acids, and neutral salts, such as sodium acetate.

Seliwanoff's Test.

This test has been advanced as one characteristic for the ketoses in distinction from the aldoses. Ten c.c. of urine are treated with a few crystals of resorcin and 5 c.c. of concentrated HCl. If the mixture be warmed a brilliant red color appears in the presence of a ketone (levulose) while no coloration is observed with an aldehyd (glucose).³ Müller has shown that glucosamin gives this test, while R. and O. Adler find a reaction in the presence of nitrous acid. If the mixture be heated too strongly or too long, mannose and maltose may also give a positive test. Adler finds that the use of acetic acid with a trace of HCl gives better results than HCl alone. It will be seen, therefore, that this test is not so characteristic as was believed, but it serves to distinguish levulose from glucose, which is the important point.

If the red solution formed in this reaction be neutralized with sodium carbonate and extracted with amyl alcohol or, preferably, with acetic ether (Borchardt), the extract will have a yellow color with a faint green fluorescence and becomes rose-red on the addition of alcohol. The spectrum of this solution shows a sharp line in the green between *E* and *b*, while if the solution be quite concentrated a second weaker line will be seen in the blue at *F*.

Methyl-phenylhydrazin Test.

Neuberg⁴ has definitely shown that fructose forms a characteristic osazone

¹ Reiss and Jehn, *Deutsch. Arch. f. klin. Med.*, 1912, CVIII, 187; Roubitschek, *Ibid.* 226; Foster, *Am. Jour. Med. Sc.*, 1912, CXLIII, 830; Editorial, *Jour. Am. Med. Assn.*, 1913, LX, 287; Bloomfield and Hurwitz, *Bull. Johns Hopkins Hosp.*, 1913, XXIV, 375; Hurwitz and Bloomfield, *Ibid.*, 380; Isaac, *Ztschr. f. physiol. Chem.*, 1914, LXXXIX, 78; Wagner *Ztschr. f. klin. Med.*, 1914, LXXX, 174; Hatiegan, *Wien. klin. Wchnschr.*, 1914, XXVII, 358; Fleckseder, *Ibid.*, 475; Arai, *Deutsch. med. Wchnschr.*, 1914, XL, 792; Wörner and Reiss, *Ibid.*, 907; Maliwa, *Med. klin.*, 1914, X, 762; Friedman and Strouse, *Arch. Int. Med.*, 1914, XIV, 531; Hoffmann, *Ztschr. f. exper. Path. u. Therap.*, 1914, XVI, 337; Schede, *Jahrb. f. Kinderhke.*, 1915, LXXXII, 45; Pari, *Gaz. d. Osp.*, 1916, XXXVI, 1217; Foster and Kahn, *Jour. Lab. and Clin. Med.*, 1916, II, 25.

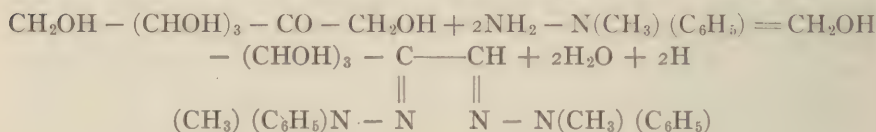
² See Vosburgh, *Jour. Am. Chem. Soc.*, 1920, XLII, 1696.

³ Königsfeld (*Biochem. Ztschr.*, 1912, XXXVIII, 310) shows that the HCl must not have a final concentration greater than 12.5 per cent. and that the heating should not be longer than 20 to 30 seconds. If dextrose, also, be present in amount greater than 2 per cent., the possibility of its conversion into levulose must be remembered. See, also, Jolles, *Ibid.*, 1912, XLI, 331.

⁴ *Ztschr. f. physiol. Chem.*, 1902, XXXVI, 227; *Ber. d. d. chem. Ges.*, 1902, XXXV, 959; *Ibid.*, 1904, XXXVII, 4616.

with methylphenylhydrazin, while no such compound is obtained with glucose, mannose, or glucosamine. The test is, therefore, the most reliable and scientific one for the presence of levulose, although it is not clinically so acceptable as the Seliwanoff reaction.

The formation of this osazone occurs according to the following equation.



Technic.

The urine is acidified with acetic acid and boiled to remove albumin if present. The mixture is then filtered and the clear filtrate, which must be acid, is evaporated in a vacuum, at a temperature not over 40°, to a thin syrup. The reaction must remain acid during the evaporation so that a drop of acetic acid may be added if necessary. The residue is thoroughly extracted with 98 per cent. alcohol, using an amount of alcohol equal to one-half the original volume of urine. Filter and re-extract the residue with alcohol should any reducing action be observed in it. The alcoholic extracts are mixed and decolorized with animal charcoal. A portion of the extract is examined for its sugar-content by Fehling's test, all of the reduction being attributed to levulose. Methylphenylhydrazin is then added to the alcoholic solution, which should not measure over 30 c.c. The amount of the hydrazin to be added is in the proportion of 3 molecules for 1 of levulose or, in other words, for each gram of sugar a trifle over 2 grams of methylphenylhydrazin. The mixture is allowed to stand for a few hours in the cold and filtered if a precipitate forms. The filtrate is treated with 50 per cent. acetic acid, using the same amount of acid as of the methylphenylhydrazin, and sufficient alcohol is added to give a clear solution. The mixture is heated from three to five minutes or, preferably, allowed to stand at 40°C., for 24 hours in an incubator. Crystals will usually separate out at the end of this time, but if not they will appear on the addition of a few drops of water. The crude product is purified by recrystallization either from a mixture of chloroform and petroleum ether or from hot water to which pyridin has been added. The yield by this method is 81 per cent. of the total sugar if pure solutions are used, while from urine it is but 50 per cent.

The crystals of *methylphenylosazone* are delicate yellow, long, fine, needles melting from 158 to 160°C. This method can hardly find application in the hands of the general practitioner.

Pure levulosuria is recognized by the levorotation of the urine, which possesses reducing properties and is capable of fermenting with yeast. After fermentation, the urine loses its reducing and optical properties. The presence of a levulosuria is indicated by a considerable difference between the results obtained by titration and polarization, providing a glycosuria be coexistent. The Seliwanoff reaction should be used as a routine in every case which shows both fermentation and reduction. The general practitioner

will rarely have access to a polariscope and even then may be misled if other interfering substances, such as albumin, glycuronic acid, and β -oxybutyric acid be present. Do not presuppose that glucose is the only fermentable and reducing sugar of the urine, as the recognition of the true condition may make much difference in the treatment as well as in the prognosis.

(c) **Pentose.**

The pentose¹ group of carbohydrates comprises eight possible stereoisomers with the general molecular formula of $C_5H_{10}O_5$. Three members of this group, rhamnose, fucose, and chinovose, are substituted pentoses, viz., methyl pentoses having the formula $C_6H_{12}O_5$. These latter are the exceptions to the general rule that carbohydrates contain the same number of carbon atoms as of oxygen atoms, and have led to the more scientific method of classifying the carbohydrates according to the number of oxygen atoms rather than of carbon atoms. The pentoses are widely spread throughout the vegetable kingdom in the form of their anhydrides or in combination with other groups of atoms. They are also found as constituents of the nucleoproteins of animal tissues, being especially abundant in the pancreas.

It has been found that the ingestion of large amounts of pentose-containing food, such as apples, cherries, plums, beets, and the leguminous vegetables, leads to the excretion of pentose in the urine. This *alimentary type of pentosuria* is characterized by the presence of optically active xylose or arabinose and appears after the ingestion of small amounts, in some cases following an intake of as low as 50 mg. of the pure carbohydrate. In true diabetes mellitus the urine frequently contains l-xylose which probably arises from the breaking down of the pancreatic nucleoprotein. Alfthan states that the pentoses are constantly present in diabetic urine, so that it is highly probable that these sugars might be found whenever search was made for them in diabetic conditions. It is not strange that these carbohydrates are not more frequently reported, as their reducing action leads to confusion unless controlled by fermentation methods. The excretion of pentoses in diabetic conditions is not necessarily increased in direct proportion to the intake, as their absorption may be so slow that accumulation is not possible before oxidation has occurred.

Salkowski and Jastrowitz² reported in 1892 the finding of a pentosuria which was not associated with intake of pentose food nor with diabetes mellitus. This type is known as *idiopathic, essential, or intrinsic pentosuria*, of which 24 cases were found by Janeway³ up to 1906. The peculiar thing of this type of pentosuria is that the sugar excreted is r-arabinose, an optically inactive pentose. This is the single exception in which an optically inactive pentose is found in all nature. This fact characterizes this type of pentosuria as an anomaly of metabolism *sui generis*. The origin of this urinary pentose is still unsettled. The source must be within the organism, as no inactive arabinose is taken as food, and if it be given in experimental cases

¹ See Bendix, *Die Pentosuria*, Stuttgart, 1903; Rockwood and Khorozian (*Jour. Biol. Chem.*, 1921, XLVI, 553) state that the limit of xylose by animals is low.

² *Centralbl. f. d. med. Wissensch.*, 1802, 337.

³ *Am. Jour. Med. Sc.*, 1906, CXXXII, 423. See, also, Cammidge and Howard, *Brit. Med. Jour.*, 1920, II, 777; Justin-Mueller, *Jour. pharm. chim.*, 1991 (7-s), XXIII, 317.

it appears in the urine as d-arabinose. Moreover, it cannot be derived from the nucleoprotein as the pentose in these cases is l-xylose.¹ Neuberg suggests that galactose might be considered the source of this pentose, but no proof of this has been forthcoming.

In the true idiopathic pentosuria the assimilation of other carbohydrates is unchanged and does not influence in any way the excretion of r-arabinose, although the active types of this pentose may be excreted at the same time in the urine. It is interesting to find that the pentoses taken in as food are excreted in different proportions by the diabetic and nondiabetic subjects. Thus von Jaksch observes that diabetics excrete from 49 to 82 per cent. of arabinose of the food and nondiabetics 1 to 47 per cent., while nondiabetics excrete from 19 to 55 per cent. of xylose and diabetics only a trace. The amount of pentose excreted in essential pentosuria has been reported as varying between 0.08 and 1 per cent. Neuberg has recently shown that a certain amount of the r-arabinose is combined with urea in the form of a ureid, which does not reduce Fehling's solution until it undergoes hydrolysis with acid. For this reason he believes that the amount of pentose reported is in practically all cases 100 per cent. too low. Luzzatto² has reported the excretion of the optically active l-arabinose entirely independent of the food intake.

The pentoses reduce copper solutions as do other carbohydrates, but the reduction is much slower, appearing during the cooling of the fluid. Five c.c. of Folin's solution are reduced by 0.0271 gram of pentose. They do not ferment with yeast and do not give a typical reduction with the Almen-Nylander test, the color being a gray rather than a black. In the true idiopathic pentosuria no reaction is observed with the polariscope, while in the alimentary type a slight dextrorotation is usually noted, although von Jaksch reports the excretion of an inactive arabinose after the ingestion of active pentoses. These pentoses form more or less typical osazones which melt between 157 and 160°, but the reaction is not so easily produced.³ These pentosazones are readily soluble in warm water and show dextrorotation. The most characteristic chemical property of these types of carbohydrate is the formation of furfural ($C_2H_2O_2$) when they are distilled in the presence of acids. The color reactions given below are based upon the production of furfural and the formation of distinct colorations on treatment with various reagents.

Tollen's Test.

A few c.c. of concentrated hydrochloric acid are saturated with phloroglucin, care being taken to leave a small amount undissolved. This solution is then divided into two equal parts, to one of which is added 1/2 c.c. of the suspected urine and to the other 1/2 c.c. of normal urine. Both tubes are then

¹ Elliott and Raper (Jour. Biol. Chem., 1912, XI, 211; Ibid., 1913, XV, 481) suggest the possibility of this sugar being ribose.

² Beitr. z. chem. Physiol. u. Path., 1905, VI, 87. Robertson (Jour. Am. Med. Assoc., 1920, LXXV, 51) reports a case in which the patient excretes this optically active arabinose with no trace of glucose and in which there is markedly lowered resistance to staphylococcal infections.

³ Klercker, Deutsch. Arch. f. klin. Med., 1912, CVIII, 277; Levene and La Forge, Jour. Biol. Chem., 1913, XV, 481; Ibid., 1914, XVIII, 319; Rosenbloom, Jour. Am. Med. Assn., 1915, LXIV, 508; Zlataroff, Ztschr. f. physiol. Chem., 1916, XCVII, 28; Hiller, Jour. Biol. Chem., 1917, XXX, 129; Fred, Peterson, and Davenport, Ibid., 1919, XXXIX, 347.

placed in a boiling water-bath for a few minutes, when an intense red zone will appear in the upper portion of the tube if pentose be present. After a few moments this red color will gradually spread throughout the fluid, while the control urine shows no marked change in color. It is advisable to remove the tubes from the water-bath as soon as the color appears, as the clearness of the reaction is interfered with by prolonged heating. The coloring matter is then extracted by shaking with amyl alcohol, when spectroscopic examination will show an absorption band between *D* and *E*.

This test reacts in the same way with glycuronic acid so that it has little value in differentiating pentose from the former substance. As a rule, the free glycuronic acid is not so easily split from its conjugated compound as is furfural from pentose, so that the test is at least suggestive of pentose.

Orcin Test.

For this test the urine should be decolorized by heating with animal charcoal and filtering. Five c.c. of urine are treated with an equal volume of concentrated hydrochloric acid and a few crystals of orcin are added. The mixture is then warmed approximately to the boiling-point, when a dark green color appears in the presence of pentose or glycuronic acid. The formation of a greenish-blue precipitate is very strong evidence of pentose rather than glycuronic acid. The pigment is then extracted with amyl alcohol, when spectroscopic examination shows a characteristic absorption band between *C* and *D*. The presence of glucose may interfere with the reaction, so that it may be necessary to remove it by fermentation.

Bial has modified this test in such a way that glycuronic acid is less apt to be a disturbing factor. His reagent consists of 500 c.c. of 30 per cent. HCl to which are added 1 gram of orcin and 25 drops of 10 per cent. ferric chlorid solution. Four to five c.c. of this reagent are heated to boiling and removed from the flame. The suspected urine is then added drop by drop, not exceeding 1 c.c. in all, when a green color should appear almost immediately if pentose be present. The heat employed is hardly sufficient to split off glycuronic acid. Here also glucose, if present, should be removed by fermentation with a pure culture of yeast rather than with compressed yeast, as the bacteria possibly present in yeast may break up the pentoses at the same time.

The osazone may be formed as previously given under Glucose. The glucosazone is separated from the pentosazone by digesting with water not over 60° in temperature, the pentosazones being dissolved. If the pentosazone be treated with 20 c.c. of water and 5 c.c. of concentrated hydrochloric acid and distilled, the distillate will give a beautiful test with *Bial's* reagent, which absolutely eliminates glycuronic acid and other interfering substances possibly present in diabetic urine.

Quantitative Determination.

Neuberg and Wohlgemuth¹ have introduced a method by which the arabinose of the urine may be accurately determined. A preliminary determination of the sugar present is made by Purdy's solution, eliminating glucose by previous fermentation. If less than 1 per cent. of reducing sugar, which is

¹ Ztschr. f. physiol. Chem., 1902, XXXV, 31 and 41.

assumed to be arabinose, is present, the urine must be concentrated in a vacuum so that the sugar content is slightly over 1 per cent.

Technic.

One hundred c.c. of urine are acidified with two drops of 30 per cent. acetic acid and evaporated on a water-bath to approximately 40 c.c. It is then treated with 40 c.c. of 96 per cent. alcohol, the mixture is allowed to stand for two hours, and is then filtered from the separated urates and inorganic salts. The residue is carefully washed with 40 c.c. of 50 per cent. alcohol. To the filtrate 1.4 grams of pure diphenylhydrazin are added and the mixture heated on a boiling water-bath for one-half hour, the alcohol being replaced as it evaporates. The mixture is allowed to stand for 24 hours and is filtered through a Gooch filter, using the mother liquor to transfer the precipitate. The crystals are then washed with 30 c.c. of 30 per cent. alcohol, and the Gooch with its contents dried at 80°C. to constant weight. The amount of arabinose is obtained by multiplying the weight of the diphenylhydrazone by 0.4747 or by dividing by 2.107.

Cambridge's Reaction.

Cambridge¹ has found that the urine in cases of pancreatic disease contains a substance which gives an osazone when treated with phenylhydrazin. In his earlier work he was led to believe that this substance was possibly glycerin or a derivative. He advanced two reactions, the first of which he found to be due to a mixture of glycuronic acid and a true carbohydrate, while the second was apparently due only to the glycuronic acid. In his later studies on this reaction he was led to believe that the mother substance of the osazone was a pentose (probably xylose) split off from a dextrin-like body by the hydrolysis with hydrochloric acid. This view has been somewhat substantiated by others. The careful work done in recent years by Kinney,² Whipple and King,³ Whipple, Chaffer and Fischer,⁴ Swan and Gilbridge,⁵ Wilson,⁶ and Roper and Stillman⁷ has proven conclusively that, although this reaction is given in some cases of pancreatitis, it is not pathognomonic as it occurs in many other conditions and may not, even, be present in pancreatic disease.⁸ This test is not to be regarded, therefore, as of much diagnostic value.

Technic.

The writer gives only the improved method known as "Reaction C" referring to the original work of Cambridge for reactions A and B. The urine to be tested should be a portion of the 24-hour specimen and must be freed from glucose and albumin by methods previously outlined.

¹ *Lancet*, 1904, I, 782; *Ibid.*, 1905, II, 14; Robson and Cambridge, *Surgery of the Pancreas*, London, 1907. See Cambridge and Howard, *Lancet*, 1914, II, 791; Pikelhering and van Hoogenhuyze, *Ztschr. f. physiol. Chem.*, 1914, XCI, 151; Lameris and van Hoogenhuyze, *Nederl. Tijdschr. v. Geneesk.*, 1914, II, 675.

² *Am. Jour. Med. Sc.*, 1910, CXL, 878.

³ *Bull. Johns Hopkins Hosp.*, 1910, XXI, 196.

⁴ *Ibid.*, 1910, XXI, 339.

⁵ *New York Med. Jour.*, 1910, XCI, 781.

⁶ *Trans. Assoc. Amer. Phys.*, 1910.

⁷ *Arch. Int. Med.*, 1911, VII, 252.

⁸ See Mayesima, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1912, XXV, 403; also, Karas, *Ztschr. f. klin. Med.*, 1913, LXXVII, 125.

Forty c.c. of clear, filtered acid urine are acidified with 2 c.c. of concentrated HCl and boiled for 10 minutes. The mixture is then cooled and made up to 40 c.c. with distilled water. The excess of acid is then neutralized by the addition of 8 grams of lead carbonate and the mixture cooled if necessary. Filter off the resulting precipitate and treat the filtrate with 8 grams of powdered tribasic lead acetate to remove the glycuronic acid. Filter, treat filtrate with 4 grams of powdered sodium sulphate, heat to the boiling-point, and allow to cool. The lead sulphate is removed by filtration. Ten c.c. of the clear filtrate are made up to 17 c.c. with distilled water, 0.8 gram of phenylhydrazin, 2 grams of sodium acetate, and 1 c.c. of 50 per cent. acetic acid are added and the mixture boiled for 10 minutes. Filter while hot and make the filtrate up to 15 c.c. with warm water. The mixture is allowed to cool, when yellow crystals arranged in sheaves and rosettes may be observed under the high-power lens.

(d) **Lactose** ($C_{12}H_{22}O_{11}$).

Lactose is found in the urine of women during the period of lactation and may be found in patients who have been on an exclusive milk diet for a long period. A distinct type of alimentary lactosuria is observed on account of the low assimilation limit for milk-sugar. In breast-fed children with gastrointestinal disturbance lactose associated with galactose may be found in the urine. In this case Langstein and Steinitz¹ have shown that the excretion is not due to failure of the normal enzyme, but to an unknown derangement of the activity of lactase, which renders it incapable of splitting up the whole of the lactose, the remainder being absorbed unchanged. From the portion which is split up in the bowel the resulting easily assimilable glucose is utilized by the organism, while galactose partly escapes by the kidneys, on account of the much lower assimilation limit for this latter carbohydrate. Lactose in these cases is usually associated, therefore, with galactose (Neuberg). According to the work of Voit, an increase of lactose in the diet of a diabetic is associated with an increased output of glucose.

The usual form of *lactosuria* is that observed in the parturient female. It is ordinarily first seen a few days after delivery of the child, but occasionally appears during the latter days of gestation, as Ney, Lemaire, and Porcher have shown. The amount of lactose excreted by the nursing mother equals 2 to 3 per cent., according to Naunyn, while McCann places the average at 0.35 per cent. for the first few days of the puerperium. Lactose may continue in the urine for some time, the actual amount depending upon the quantity of milk as well as its quality.² If nursing is interrupted for any reason, more lactose will be found than when nursing is regular.

Lactose reduces copper solutions, although somewhat less actively than does glucose. It also shows a positive Almén-Nylander reaction. It has a

¹ Beitr. z. chem. Physiol. u. Path., 1906, VII, 575. Schloss (Am. Jour. Dis. Child., 1921, XXI, 211), while not denying that alimentary lactosuria may occur in cases of toxicosis of children, believes that it is at most a clinical rarity in comparison with the frequently observed exaggerated excretion of glucose (glycuresis).

² See Grönvall, Biochem. Ztschr., 1912, XL, 145; Rosenbloom (Jour. Am. Med. Assn., 1915, LXIV, 508) reports the finding of lactose in the urine of an adult male after intake of milk.

strong rotatory power, its specific rotation being practically the same as that of glucose (+ 52.5°). It does not ferment with yeast, although bacteria if present may hydrolyze it into its constituents, glucose and galactose, the former of which will show fermentation. It is, therefore, advisable when applying the fermentation test not to judge of a reaction which has progressed longer than a few hours. With phenylhydrazin it forms a lactosazon which appears in the form of sheaves of delicate curved needles much resembling bunches of yellow thread. These crystals melt at 200°C. The test is not easily obtained unless the urine be concentrated to a small bulk and the residue extracted with alcohol, when the alcohol is evaporated and this residue taken up with water and the phenylhydrazin test then applied.

Rubner's Test.

Ten c.c. of urine are treated with an excess (3 grams) of lead acetate and boiled for a few minutes.¹ The yellowish or brown solution is then filtered and ammonia added to the filtrate until a slight permanent precipitate remains. An intense brick-red fluid is obtained which later shows the deposition of a cherry-red precipitate with a colorless supernatant fluid. This test is not very delicate as it shows lactose only when present in amounts varying from 0.3 to 0.5 per cent. Glucose gives with this test a red solution, but a more distinctly yellow precipitate.²

Lactosuria is to be assumed when the urine possesses reducing properties and dextrorotation, but is incapable of fermenting with ordinary yeast within 12 hours. If the urine be boiled with 2 per cent. sulphuric acid and then neutralized, its optical activity will be increased and it will be capable of undergoing fermentation. It is to be remembered in testing for the amount of lactose by the use of Folin's or Purdy's solutions that 5 c.c. of the former and 35 c.c. of the latter are reduced by 0.0339 and 0.02712 gram respectively.

(e) Maltose ($C_{12}H_{22}O_{11}$).

Maltose has occasionally been reported in the urine, although many of the cases are questionable as the proper identification of the sugar was not thoroughly carried out. The most reliable cases appear to be those of Noble, von Ackeren, Rosenheim and Flatow, and especially that of Magnus-Levy. In this latter case the urine showed a considerable excess of rotation when compared with its reducing power. After inversion with dilute acid, by which each molecule of maltose was converted into two molecules of glucose, the rotation diminished and the reduction increased, so that the polarimetric and titration methods gave concordant results. The urine underwent complete fermentation with synchronous loss of optical activity and of reducing power. Calculations founded on these determinations showed that 1.5 per cent. of maltose and 2 per cent. of glucose were present. This seems to be a case in which the amount of maltose excreted exceeds all records (Neuberg). The cases in which maltose appears in the urine seem to be those of disease of the pancreas, especially those with interstitial lesions.

Maltose reduces copper solutions, but not as strongly as does glucose.

¹ See Cole, *Biochem. Jour.*, 1914, VIII, 134; also, Rogerson, *Ibid.*, 1915, IX, 245.

² For other tests see Adriano, *Philippine Jour. Sc.*, 1920, XVII, 213; Herzberg, *Biochem. Ztschr.*, 1921, CXIX, 81.

Five c.c. of Folin's and 35 c.c. of Purdy's solution are completely reduced by 0.04035 and 0.03228 gram respectively of maltose. It is much more strongly dextrorotatory than glucose and forms an osazone which crystallizes in large prism-like needles arranged in sheaves, and melts at 207°C. This osazone is soluble in water and shows a dextrorotation, it being more distinctly identified by determination of the nitrogen content, which should equal 10.6 per cent. Maltose ferments with yeast only after inversion by heating with acid, the splitting products being two molecules of glucose.

Other carbohydrates, such as dextrin, isomaltose, and saccharose, have been reported in the urine. These are extremely rare and need little comment in this place. In the case of cane-sugar the assimilation limit is so high that an alimentary saccharosuria could occur only after an enormous intake. Spontaneous excretion of cane-sugar has never been actually proven, but this sugar may be found in the urine of hysterical patients who have added it to deceive the physician. The so-called *animal gum*, first isolated by Landwehr, seems to be a normal constituent of urine. Alfthan¹ finds it is present in practically every case of diabetes to the extent of 1 to 37 grams per diem. This substance is probably not a definite chemical body, but a mixture of several. Little is known of its chemistry.

Inosite was regarded for a long time as a carbohydrate, but it is now known to be a hexaoxyhexahydrobenzol with the formula $C_6H_6(OH)_6$. This substance, has, therefore, nothing to do with true carbohydrate metabolism, but is discussed at this point as it has so long been regarded in this connection. Inosite enters into the composition of almost all animal tissues and occurs both in the optically active and inactive forms. A physiologic excretion of inosite is not infrequent, according to Hoppe-Seyler. It may occur in nephritis, diabetes mellitus and insipidus, and after a large intake of animal food.²

(f) **Glycuronic Acid** ($CHO-(CHOH)_4-COOH$).

Glycuronic acid is an intermediate product of the oxidation of carbohydrate, the CH_2OH group being converted into CHO while the original CHO group is oxidized into $COOH$. This acid still retains the aldehyd group, in consequence of which it shows the same reducing action as does glucose. It seems to be characteristic of glycuronic acid that, when produced naturally, it is never found in the free state, but only in the combined form as the conjugated glycuronic acid. It seems to be especially capable of combining with substances showing alcoholic or phenolic characteristics.³ The free glycuronic acid may be split off from its conjugated compounds by heating with acid and other hydrolyzing agents. The conjugated glycuronates are levorotatory while the free acid shows dextrorotation. Among the substances with which glycuronic acid combines we find chloral hydrate, butyl chloral hydrate, chloralamid, camphor, menthol, carbolic acid, resorcin, acetanilid, antipyrin, phenacetin, pyramidon, sandal-oil, morphin and

¹ Ueber dextrinartige Substanzen in diabetischen Harn, Helsingfors, 1904. Cammidge and Howard, *Lancet*, 1914, II, 791.

² See Anderson and Bosworth, *Jour. Biol. Chem.*, 1916, XXV, 399; Greenwald and Weiss, *Ibid.*, 1917, XXXI, 1. See OKey (*Jour. Biol. Chem.*, 1919, XXXVIII, 33 and XXXIV, 149) for a study of the behavior of inulin in the animal body.

³ See Hämäläinen, *Skand. Arch. f. Physiol.*, 1913, XXX, 196.

cocain. The normal metabolism following intake of any of these substances is such that excretion of conjugated glycuronic acids will follow and may, therefore, lead to the assumption of sugar in the urine unless precautions are taken properly to differentiate these compounds. Besides conjugated glycuronic acid of the above type we find a combination of urea with glycuronic acid as well as a certain amount of indoxyl, skatoxyl, phenol, and cresol in combination with this acid. Most of the products of bacterial decomposition in the intestine are excreted in combination with sulphuric acid, but some is invariably present as a conjugated glycuronate.

The origin and formation of glycuronic acid within the system is not entirely understood. It has been supposed to be derived from protein as especially advocated by Loewi, but Mayer has rather disproven Loewi's work, and shows that probably glycuronic acid is a direct derivative of glucose and that all carbohydrate oxidation must pass through the intermediate stage of glycuronic acid.

The status of this question is very well summed up by Neuberg as follows: The formation of glycuronic acid out of protein is by no means excluded, nor yet from fat; but as it is difficult to eliminate the direct formation of glycuronic acid or its secondary development from previously existing grape sugar, Mayer justly contends that the question of glycuronic acid formation from these substances is practically included in the broader question of the formation of sugar from fat and protein.

Chiray and Caille¹ believe that important information as to hepatic function may be obtained from the appearance of glycuronic acid in the urine after the patient swallows, fasting, two capsules each containing 0.5 gram of camphor. The urine is collected at once and again six hours later. This is the "induced glycuronic acid test."

The exact point of conjugation of glycuronic acid is unsettled. It has been assumed by some to occur in the liver, while others find that the liver plays no part. It is probable that the synthesis takes place in various parts of the organism.

It has been shown that the output of glycuronic acid may be increased in diabetes mellitus, in mild cases the unoxidized sugar being present largely in this form. Mayer² advances the hypothesis of incomplete oxidation of sugar to explain its appearance in these cases.³ He shows that after the administration of glucose in amounts beyond the assimilation limit, an occasional excretion of glycuronic acid occurs with an equivalent diminution of the ethereal sulphates. It is possible that the substance conjugating with glycuronic acid is unknown and that we have the same results as though similar substances were introduced by mouth. As Mayer has advanced no direct proof of the correctness of his theory, the decision must be left for the future. Clinically, the question of the highest importance is whether the excretion of glycuronic acid is of any diagnostic value and whether it

¹ Bull. de la soc. méd. des Hôp. de Paris, 1921, XLV, 383.

² Ztschr. f. physiol. Chem., 1901, XXXII, 518; Berl. klin. Wchnschr., 1903, XL, 292 and 514. See Biberfeld, Biochem. Ztschr., 1914, LXV, 479; Roger and Chiray, Bull. l'Acad. de Med., 1915, LXXIII, 444.

³ See Conzen, Ztschr. f. klin. Med., 1912, LXXV, 426.

is of prognostic significance in diabetes. It does not seem wise to assume that a patient showing an occasional increase in the glycuronic acid excretion, which cannot be accounted for by intake or increased production of conjugating substances, will in the future show typical diabetes. Edsall does not believe in the value of glycuronic acid in the diagnosis of a latent diabetes, nor does Neuberg regard an increased excretion of glycuronic acid as the original derangement which may determine other deviations from health.¹

Very few of the conjugated glycuronates show reducing action when treated with copper solutions using the precautions previously laid down. The chloral and camphor compounds are much more apt to produce typical reduction, but even these require heating for somewhat longer periods than does glucose. A diagnosis of a glycuronic acid excretion is based upon the following points: The fresh urine is levorotatory, but shows little or no reducing properties and does not ferment. This same finding will be observed if β -oxybutyric acid be present so that a diagnosis may not rest on these findings alone. After being boiled with dilute acid for a period varying from one-quarter to three-quarters of an hour, the levorotation is changed to dextrorotation and the urine shows strong reducing powers. Such tests will not be given by β -oxybutyric acid. In some cases after heating the urine with acid the action upon light may remain levorotatory or the solution may be optically inactive, on account of the fact that the conjugating substance may be levorotatory or that complete hydrolysis has not been effected. On heating the urine for some time with Bial's modification of the orcin test a positive reaction appears with the liberated glycuronic acid. This acid crystallizes with phenylhydrazin forming distinct yellow needles which melt at 114 to 115°C. This test is, however, not readily obtained so that it is difficult to identify glycuronic acid by its osazone. It will be seen, therefore, that glycuronic acid is differentiated from the pentoses largely by its levorotation when in the conjugated state or dextrorotation when free.

Tollens (Ber. d. chem. Ges., 1908, XLI, 1788) has advanced a test which permits of a clear differentiation between glycuronic acid and pentose. To 5 c.c. of urine add $\frac{1}{2}$ c.c. of a 1 per cent. alcoholic solution of naphthoresorcin and 5 c.c. of concentrated HCl. Warm over the free flame to boiling or place in the boiling water-bath for 15 minutes. Allow to stand for 4 minutes and then cool under running water. Add an equal volume of ether and shake vigorously. On separating, the ether layer shows, in the presence of glycuronic acid, a violet to blue coloration. Spectroscopic examination reveals a sharp absorption band at the D line. The test is not given by pentose. (See Tollens, Ztschr. f. physiol. Chem., 1908, LVI, 115; Jolles, Ibid., 1912, LXXXI, 203; also, Neuberg and Schewket, Biochem. Ztschr., 1912, XLIV, 502.)

¹ See Medigreceanu (Jour. Exper. Med., 1913, XVIII, 259) for a discussion of the glycuronic acid excretion in pneumococcus infections. Roger, C. R., Soc. biol., Paris, 1915; LXXVIII, 714; Jean, Arch. Mens d'Obs. et de Gyn., 1915, IV, 383; Roger and Chiray. Gaz. Med. de Paris, 1915, LXXXVI, 46; Barbier, Arch. d. Med. des. Enf., 1916, XIX, 225; Roger, Arch. d. Mal. de l'App. Dig., 1918, IX, 61; Presse Méd., 1916, XXIV, 217; Gautier, Ibid., 339; Pi Suñer, Siglo Med., 1917, LXIV, 146; Chiray, Paris Méd., 1919, IX, 359, Weber, Paris Méd., 1920, X, 472; Lorenzani, Policlinico, 1920, XXVII, 174; Azzi, Rif. Med., 1920, XXXVI, 629; Coda, Ibid., 1922, XXXVIII, 32.

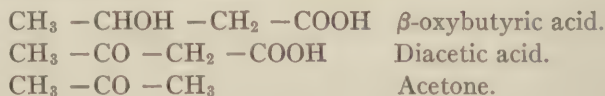
*Goldschmiedt*¹ has introduced a test which *Mayerhofer*² believes of great value to the pediatrician, as it indicates intestinal derangement better than indican. One-half to 1 c.c. of urine is treated with 2 drops of a 15 per cent. alcoholic solution of α -naphthol. Carefully overlay 3 to 4 c.c. of concentrated H_2SO_4 with this mixture. A violet ring is observed, which changes to a distinct emerald green on standing. In this test the urine must be free from nitrites and nitrates.

Neuberg's Test.³

Five hundred c.c. of urine are treated with sufficient sulphuric or phosphoric acid to make the acidity from 1 to 2 per cent. This acidified urine is then heated in an autoclave for two hours at a temperature of 115°C . The mixture is then cooled, neutralized with sodium carbonate, acidulated with acetic acid, and filtered. Two hundred and fifty c.c. of the filtrate are mixed with a hot aqueous solution of 5 grams of parabromophenylhydrazin hydrochlorate and 6 grams of sodium acetate. The mixture becomes cloudy at once, but on heating the cloudiness will disappear. As the mixture cools needle-shaped crystals will separate out and may be filtered off, the filtrate being again heated and cooled to obtain more crystals. This process may be repeated until no more crystals form. These crystals are then washed with distilled water followed by absolute alcohol, and are then recrystallized by dissolving in 60 per cent. alcohol and gradually evaporating. They are clear yellow in color, melt at 236°C ., and show marked levorotation when dissolved in a mixture of pyridin (4) and absolute alcohol (6). This test is distinctive

(3) Acetone Bodies.

By the acetone bodies⁴ we mean acetone, diacetic acid and β -oxybutyric acid. The latter of these is the mother substance so that this group would better be called the β -oxybutyric bodies. The chemical relation between these bodies is very close, the β -oxybutyric acid being oxidized to diacetic acid, which then splits up into acetone and carbon-dioxid. This may be seen from the following formulæ:



Formerly these substances were supposed to be derived from protein material, the β -oxybutyric acid being formed from the β -amino acids by desamidization and oxidation in the β position. This theory is, however, not generally held at present, being replaced by the more modern idea that the fats are the chief source of the acetone bodies. It has been found that in perfectly sound, well-nourished individuals the addition of fat causes only a very slight in-

¹ Ztschr. f. physiol. Chem., 1910, LXV, 389; 1910, LXVII, 194. Pabatani, Policlinico, 1920, XXVII, 149; Diebschlag. Arch. path. Anat., 1921, CCXXX, 179.

² Ztschr. f. physiol. Chem., 1911, LXX, 391.

³ Ber. d. d. chem. Ges., 1910, XXXII, 2395; Ztschr. f. physiol. chem., 1905, XLIV, 127.

⁴ See Waldvogel, Die Acetonkörper, Stuttgart, 1903; Magnus-Levy, Ergebnisse der klin. Med., Jena, 1908; Lepine, Rev. de Med., 1913, XXXIII, 601; Marriott, Jour. Biol. Chem., 1914, XVIII, 241.

crease in the output of acetone bodies and, strangely enough, that butyric acid itself causes no acetonuria. On the other hand in normal individuals from whom the dietary carbohydrate has been removed or in a diabetic who is not utilizing what carbohydrate he may be allowed, a marked excretion of acetone bodies may occur. While a portion of this acetone may possibly be derived from the carbohydrate groups of the protein molecules, it can hardly explain the enormous excretion in diabetes, as the amount of protein catabolism, as shown by the urinary nitrogen, is greatly insufficient to yield any such amount of acetone bodies. The fats are, therefore, the more probable source of these bodies. As long as the body is supplied sufficient carbohydrate or is able to oxidize a sufficient amount the acetone bodies of the urine remain low; but when the system is no longer capable of oxidizing the carbohydrates, the amount of acetone bodies increases to a marked extent. We see, therefore, that the older method of allowing diabetics practically no carbohydrate food may directly lead to aggravation of the symptoms which the withdrawal was supposed to remedy. In other words, the normal or the diabetic individual must have a certain amount of carbohydrate food in order that proper metabolism may be maintained.¹ This is not the time or place to discuss the therapy or dietetic treatment of diabetes, but it must be remembered that the most successful diet is one which contains carbohydrates up to the point of tolerance. Even here we find that certain types of carbohydrates may be given to diabetics without increasing the glycosuria, while at the same time leading to a diminution in the excretion of acetone bodies.

The condition arising from a surcharging of the blood with these acetone bodies is known as *acidosis*.² (This definition applies especially to diabetes and has no reference to the acidosis of other types. A better word for this condition is ketosis.) For a long time it was supposed that the carbohydrates were not only accountable for glycosuria, but also for the acetonuria and acidosis noted in diabetes. In the advance of pathologic chemistry it has been shown that, instead of causing these latter symptoms and conditions, the carbohydrates in reality lessened them. This may be shown by the administration of a definite amount of sugar, especially in the milder types of diabetes, to patients from whose diet sugars have been previously excluded. The omission of sugar from the diet forces the organism to utilize its protein and fat and thus gives rise to an accumulation of nitrogenous and fatty metabolic products as well as to an increase in the acids of the body fluids. In this way an acidosis already present would be increased in intensity. If the carbohydrate-free diet be continued for some time, a readjustment takes place and the acetonuria may gradually diminish, as is instanced by the fact that certain races show no acetonuria even though on an absolutely carbohydrate-free diet.

With regard to the proteins as the mother substances of these bodies, we must admit that their influence is to some extent a double one.³ In the first

¹ See Blodgett, New York, Med. Jour., 1915, CII, 458.

² Folin and Denis, Jour. Biol. Chem., 1915, XXI, 183; Stern, Arch. Diag., 1915, VIII, 128; Beddard, Pembrey and Spriggs, Brit. Med. Jour., 1915, II, 389. See Straub, Deutsch. Arch. f. klin. Med., 1913, CIX, 223; also, Benedict and Joslin, Deutsch. Arch. f. klin. Med., 1913, CXI, 333. For a discussion of the newer conceptions of acidosis and of the methods of estimating the degree of acidosis see section on Blood.

³ See Pauly and Boulud, Lyon Méd., 1917, CXXVI, 118.

place protein tends to diminish the acetonuria on account of its carbohydrate content, those proteins containing the greatest number of carbohydrate groups not necessarily exerting the greatest effect either on this condition or on the glycosuria. With a diet excessive in protein the influence is, however, not of this sort. The sulphuric and phosphoric acids as well as the small amount of acetone bodies formed by the hydrolysis of the protein tend to increase an existing acidosis, while the carbohydrates formed in the splitting of these proteins may greatly increase an existing glycosuria. These points, together with the fact that the products of nitrogenous metabolism may greatly increase the osmotic tension of the blood and thus lead to disordered cell function, show us that proteins cannot be advantageous as an exclusive diet in diabetes. As is well known, the nitrogenous excretion is much more marked in a diabetic than in a nondiabetic owing to several factors. In the first place, the diabetic consumes more protein than the normal individual because his diet is limited as regards carbohydrates and must be made up to a requisite caloric value by protein and fat. Secondly, owing to the lack of the protein-sparing function of the carbohydrates, excessive protein is broken down and elaborated in order to furnish a portion of the energy necessary to maintain the body function. It must, however, be said that the diabetic protects himself for a time from the unusual loss of protein by the utilization of fat.

Concerning the fats, it is to be recalled that, although formerly accredited with no power of influencing acetonuria, to-day they are regarded as directly affecting this condition to a great extent. This is true of the fatty acids, especially of the lower members, and not of the neutral fats. If the contention of Kastle and Loevenhart be true, that a reversible action of lipase converts the fatty acids and glycerin formed by a previous hydrolysis again into neutral fats, then the influence of fats on the acidosis is variable, or else we must assume a lack of lipase in the cells of the diabetic. We know that the fatty acids belong to the ketoplastic group (substances increasing excretion of acetone bodies), yet as Borchardt has recently shown this ketoplastic action is, doubtless, due to the union of the fatty acids with glycerin, thus withdrawing from the system the antiketoplastic body, glycerin, and enabling the remaining fatty acids to exert their influence on the formation of the acetone bodies. Fats do not increase an existing glycosuria as many experiments have shown, yet we must grant that a formation of sugar from fat does take place. Von Noorden speaks of a "facultative formation of sugar from fat," referring to the fact that the demand for sugar may become so great that this source is called upon to furnish its quota of carbohydrates. We must also remember that the synthesis of fat from disintegrated carbohydrate is much affected in diabetes. Were this not the case, a large part of the sugar, reaching the blood as such, would be synthesized by the fat-forming cells and glycosuria would be diminished. Conceiving this latter function to be normal while the former is abnormal, we may readily see the close relationship between obesity and later diabetes. With an excessive diet of fat no more fat is oxidized than when the diet is low in fat. In the latter

case, the body-fat is utilized to furnish the difference, while in the former the excess is deposited in the usual fat depositories.

Besides the excretion of acetone bodies observed in diabetes, we find fever, carcinoma, inanition, lesions of the central nervous system, digestive disturbances, delayed chloroform poisoning, cases of pregnancy in which death of the fetus has occurred or in which persistent toxic vomiting is noted, cyclic vomiting, and other conditions associated with an increased output of the acetone bodies.¹ According to Mohr, most of the cases may be traceable either to limitation of carbohydrates in the food or to diminished power of utilizing them. Increased protein catabolism may play a rôle in the pathogenesis of this condition. A general statement should be that the excretion of the acetone bodies is little influenced by the amount of fat in the food of the normal individual, providing the carbohydrate content of the diet is good; but in pathologic conditions fats play a much greater rôle than do proteins in bringing about an acetonuria.

As β -oxybutyric acid is the mother substance from which the other acetone bodies are formed by oxidation, we should expect to find, as we actually do, the severest cases showing large amounts of β -oxybutyric acid and small or even no excretion of the other members of this group. As a rule, it may be said that the more acetone the less β -oxybutyric acid but this is not always the case. In the diabetic coma we usually find large amounts of the first two members of this group while acetone may be absolutely lacking, and, on the other hand, we find in some of the milder types of diabetes acetone and no diacetic or oxybutyric acids.² Dakin and Wakeman and Dakin³ have shown that the liver possesses two ferments by which the mutual interconversion of β -oxybutyric acid and aceto-acetic acid may be effected, the one being an

¹ See Fischler and Kossow, *Deutsch. Arch. f. klin. Med.*, 1913, CXI, 479; Czapski, *Arch. f. exper. Path. u. Pharmacol.*, 1914, LXXVII, 218; Zade, *Arch. f. Kinderh.*, 1914, LXIII, 1; Kerley, *Am. Jour. Dis. Child.*, 1914, VIII, 292; Peabody, *Arch. Int. Med.*, 1914, XIV, 236; Rolly, *Ztschr. f. klin. Med.*, 1914, LXXIX, 548; Bradner and Reimann, *Am. Jour. Med. Sc.*, 1915, CL, 727; Rosenbloom, *Jour. A. M. A.*, 1915, LXV, 1715; Howland and Marriott, *Bull. Johns Hopk. Hosp.*, 1916, XXVII, 63; Woodyatt, *Jour. A. M. A.*, 1916, LXVI, 1910; Chapin and Pease, *Ibid.*, LXVII, 1351; Mills and Wearne, *Psych. Bull.*, 1916, IX, 413; Thomson, *Edinburgh Med. Jour.*, 1916, XVII, 298; Higgins, Peabody, and Fitz, *Jour. Med. Res.*, 1916, XXXIV, 263; Veeder and Johnston, *Am. Jour. Dis. Child.*, 1916, XI, 291; Howland and Marriott, *Ibid.*, 309; *Ibid.*, XII, 459; Veeder and Johnston, *Ibid.*, 1917, XIII, 89; Lackner and Gauss, *Ibid.*, 209; Schloss and Stetson, *Ibid.*, 218; Means and Rogers, *Am. Jour. Med. Sc.*, 1917, XLIII, 420; Weber, *Brit. Jour. Child. Dis.*, 1917, XIV, 33; Dertil, *Soc. med. et chir.*, Bologna, 1918, VI, 289; Kertess, *Ztschr. f. physiol. Chem.*, 1919, CVI, 258; Azzì, *Rif. Med.*, 1919, XXXV, 981; Pittarelli, *Arch. farm. sper.*, 1919, XXVII, 161; Garland, *Arch. Pediat.*, 1919, XXXVI, 468; Kerley, *Ibid.*, 472; Chabanier, *Presse méd.*, 1920, XXVIII, 242; Chabannes, *Jour. pharm. chim.*, 1920, XXI, 177; Umber, *Deutsch. med. Wchnschr.*, 1920, XLVI, 761; Chace and Myers, *Jour. Am. Med. Assoc.*, 1920, LXXIV, 641; Geelmuyden, *Skand. Arch. Physiol.*, 1920, XL, 211; Widmark, *Biochem. Jour.*, 1920, XIV, 364 and 379; Pittarelli, *Rif. Med.*, 1920, XXXVI, 303; Ross, *Can. Med. Assoc. Jour.*, 1920, X, 548; Short, *Jour. Biol. Chem.*, 1920, XLI, 503; Shaffer, *Ibid.*, 1921, XLVII, 433 and 449; Briggs and Shaffer, 1921, XLVIII, 413; Shaffer, *Ibid.*, 1921, XLIX, 143; Knoepfelmacher, *Monatsschr. f. Kinderhke.*, 1921, XXI, 241; Merrin, *Brit. Med. Jour.*, 1921, II, 405; Buttenwieser, *Münch. med. Wchnschr.*, 1922, LXIX, 83; Hubbard and Wright, *Jour. Biol. Chem.*, 1922, L, 361; Wilder and Winter, *Ibid.*, 1922, LII, 393; Hubbard and Nicholson, *Ibid.*, LIII, 209.

² See McCaskey, *Jour. A. M. A.*, 1916, LXVI, 350.

³ *Jour. Biol. Chem.*, 1910, VIII, 97 *Ibid.*, 1910, VIII, 105; Fittipaldi, *Gaz. d. Osp.*, 1917, XXXVIII, 99.

oxidizing process, the other a reduction process. The acidosis may, therefore, be more a result of the latter than of the former action.

(a) **Acetone** ($\text{CH}_3\text{—CO—CH}_3$).

Chemically, acetone is dimethyl ketone. It shows, therefore, the reactions for this group of chemical compounds but is easily confused, both with the aldehyds and alcohols. The urine rarely shows typical reactions for acetone if the older tests are applied directly to the urine, so that it is necessary to distill and examine the distillate. In this process diacetic acid is split up into acetone and carbon dioxid, so that it is impossible to tell whether acetone was preformed or was produced by heating. From the clinical standpoint it is a matter of indifference, as acetone and diacetic acid are so closely related that their clinical significance is the same, acetone representing merely a further stage in the oxidation of this group of bodies. In these tests the urine must be perfectly fresh. If it is desired to eliminate the influence of the diacetic acid upon the acetone reaction, the urine may be alkalinized with sodium hydrate and extracted with pure ether. The ether removes the diacetic acid salt.

Legal's Test (Le Nobel's Test).

To a few c.c. of the urine are added a few drops of a fairly concentrated solution of sodium nitroprussid and then sodium or potassium hydrate until the mixture is strongly alkaline. A ruby-red color, later changing to yellow, appears in the presence of acetone. It will be remembered that this same test is given by creatinin, so that further modifications are necessary to permit of differentiation. If the ruby-red solution be treated with an excess of glacial acetic acid, the first red color will change into a carmine or reddish-purple color in the presence of acetone, while the same treatment with creatinin solutions yields a yellow, changing to green and finally to a blue coloration. As Le Nobel has found, ammonium hydrate does not give this reaction with creatinin, but with acetone, although the reaction is much slower in appearing. This test is given by diacetic acid, by alcohol, and by acetic aldehyd, so that it is not especially distinctive for acetone. It is perhaps better if this test is to be used at all that the urine be previously acidified and distilled, the distillate yielding a reaction which is more sensitive, according to Studer, although diacetic acid will thus be converted into acetone.

Lange¹ has modified this test as follows: To the suspected urine add $\frac{1}{2}$ c.c. of glacial acetic acid and a few drops of a freshly prepared aqueous solution of sodium nitroprussid. Mix thoroughly and overlay the mixture with 2 c.c. of concentrated ammonium hydroxid. At the point of contact a purplish-red ring is observed in the presence of acetone.

Lieben's Test.

To a few c.c. of urine² or, preferably, of the distillate are added a few drops of concentrated sodium or potassium hydrate and a few drops of a solution of

¹ Münch. med. Wehnschr., 1906, LIII, 1764. See, also, Yanagawa, Biochem. Ztschr., 1914, LXI, 256.

² Bardach (Ztschr. f. physiol. Chem., 1908, LIV, 355) and, more recently, Rosenbloom (Jour. Am. Med. Assn., 1912, LIX, 445) have shown that protein and many of its derivatives prevent the formation of the characteristic iodoform crystals. Hence the distillate should always be used.

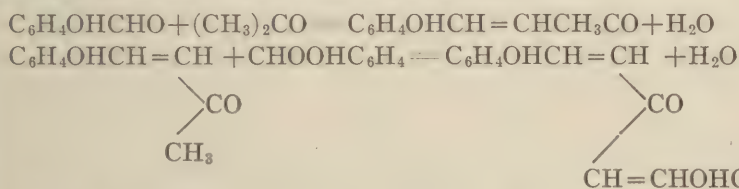
iodin in potassium iodid. On slightly warming the mixture yellow crystals of iodoform will separate, which may be recognized by their characteristic odor as well as by their hexagonal shape when examined under the microscope. This test is given by alcohol as well as by aldehyds, and will show amounts of acetone varying between $\frac{1}{100}$ and $\frac{1}{1000}$ of a mg.

Gunning's Test.

This is a modification of the previous test and is much more specific, being given only by acetone. To the distillate from the urine are added a few drops of an alcoholic solution of iodin and the mixture treated with ammonia until a black precipitate of nitrogen iodid forms. On allowing the tube to stand for periods varying between 12 and 24 hours, this black precipitate disappears, leaving a yellow sediment of iodoform, which may be recognized as mentioned above. This test is less delicate than the original one of Lieben, detecting acetone when present in amounts of $\frac{1}{100}$ of a mg. per c.c. of urine.

Frommer's Test.

Frommer¹ has introduced a test which seems to be distinctive for acetone and at the same time very delicate. It is based upon the fact that acetone reacts with salicyl aldehyd to form dioxydibenzoylacetone, according to the following equations. The alkali salt is distinctly red.



The test may be performed as follows: Ten c.c. of urine are strongly alkalinized with potassium hydrate and 10 to 12 drops of a 10 per cent. solution of salicyl aldehyd in absolute alcohol are added and the mixture warmed to about 70°C. In the presence of acetone the fluid becomes yellow, then red, later purplish-red, and, on long standing, dark red. In the absence of acetone the color of the urine is practically unchanged. This test is said by Frommer to indicate the presence of one one-millionth of a gram in 8 c.c. of urine.

Instead of applying the test as above, we may add about 1 gram of potassium hydrate (in the solid state) to 10 c.c. of urine and, without waiting for complete solution to occur, treat the mixture with 10 to 12 drops of the alcoholic solution of salicyl aldehyd and warm to 70°. At the zone of contact of the alkali and the salicyl aldehyd an intense purplish-red ring is observed only in the presence of acetone.

The writer has frequently used this test and finds it very satisfactory, as it does not react with diacetic acid unless the heating be long continued.

Quantitative Determination of Acetone.

Many of the quantitative methods given for acetone are more or less complicated and at the same time do not yield absolutely accurate results. The

¹ Berl. klin. Wchnschr., 1905, XLII, 1008. See Engfeldt, *Ibid.*, 1915, LII, 458; Csonka (*Jour. Biol. Chem.*, 1916, XXVII, 209) has introduced a colorimetric method for determination of acetone, based on this test, which is reliable and accurate but is not discussed here.

writer selects, therefore, those proving most satisfactory in his hands. All of these methods, with the exception of Folin's, give the amount of preformed acetone as well as that derived, by distillation, from the diacetic acid.¹

Huppert-Messinger Method.

The principle of this method is the determination of the amount of iodine necessary to transform into iodoform the acetone derived in the distillation of the urine. Knowing this factor, a simple calculation yields the amount of acetone present.

For this determination certain solutions are necessary:

(1) A 50 per cent. solution of acetic acid.

(2) A tenth-normal solution of sodium thiosulphate. In preparing this solution 24.8 grams of crystallized sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) are carefully weighed out and dissolved in distilled water, the solution being made in a volumetric flask and diluted exactly to the liter mark.

(3) A tenth-normal solution of iodine. This solution requires exactly 12.685 grams of iodine in one liter. As iodine is difficultly weighable on account of its volatility, it is advisable to weigh out approximately 13 grams of iodine and dissolve in approximately 1 liter of water to which has been added 25 grams of potassium iodide. This solution is then standardized by titrating against the previously made tenth-normal thiosulphate solution, using thin starch paste as an indicator and adding the iodine solution from a buret until the blue color of iodide of starch just appears. This determination is then confirmed by duplicate estimations. Twenty c.c. of the iodine solution should be the equivalent of 20 c.c. of the thiosulphate solution, so that the necessary dilution of the iodine solution may be determined by the formula previously given under Determination of Chlorides in the Urine (page 212). One c.c. of the standard tenth-normal iodine solution equals 0.012685 gram of iodine and represents 0.967 mg. of acetone.

Technic.

Five hundred c.c. of normal acid urine or 100 c.c. of acetone-rich urine are treated with 2 c.c. of 50 per cent. acetic acid for every 100 c.c. of urine and distilled² until $\frac{9}{10}$ of the volume has passed over. The distillate is collected in a receiving flask, which is cooled with ice and which contains water to absorb the acetone. The flask is tightly closed with a doubly perforated stopper, through one hole of which passes the tube of the condenser reaching below the surface of the water and through the second opening a bulb filled with water to act as a safety bulb. The tube is then washed with distilled water and the fluid in the safety bulb is emptied into the receiving flask. It is important that the distilling flask be disconnected before the heat is shut off, as otherwise the fluid might suck back. This distillate is treated with calcium car-

¹ See Sammet, *Ztschr. f. physiol. Chem.*, 1913, LXXXIII, 212; Lenk, *Biochem. Ztschr.*, 1916, LXXV, 224; Lenk and Hahn, *Munch. med. Wchnschr.*, 1917, LXIV, 179; Engfeldt, *Acta Med. Skand.*, 1919, LII, 311; Hubbard, *Jour. Biol. Chem.*, 1920, XLIII, 43; *Ibid.*, 1921, XLIX, 357; Claudius, *Hospitalstid.*, 1921, LXIV, 97; Lax, *Biochem. Ztschr.*, 1921, CXXV, 262.

² Pittarelli (*Policlinico*, 1921, XXVIII, 621) calls attention to the fact that no rubber connections should be used about the apparatus, as this may yield a volatile substance responding to the tests for acetone.

bonate to remove any nitrous or formic acid which may have distilled over, and the mixture thoroughly shaken.

This distillate is then acidified by the addition of 1 c.c. of dilute sulphuric acid (diluted eight times) and redistilled until one-tenth of the volume remains in the distilling flask. This second distillate is received in a flask arranged as in the previous distillation. It is then poured into a flask, which can be closed with a tight-fitting glass stopper. Distillate and wash-water must not fill the vessel more than one-third full. A large excess of carefully measured N/10 iodine solution is added, the mixture well shaken, and strong sodium hydrate solution added drop by drop. The flask is then stoppered, shaken for one-fourth minute, and allowed to stand for five minutes. The stopper is then removed, the fluid clinging to it washed into the flask, and the fluid acidified with strong HCl. The excess of iodine is then determined by allowing N/10 thiosulphate solution to flow from a buret until the mixture is but slightly yellow, when a few c.c. of starch paste are added and the titration continued until the blue color just disappears. One c.c. of iodine solution used by the fluid corresponds to 0.967 mg. acetone, so that all that is necessary is to subtract the number of c.c. of thiosulphate used from the number of c.c. of iodine solution added and multiply by the above factor. The result is the acetone in the amount of urine taken.

This method yields results which are from 5 to 10 per cent. low,¹ so that slight variations in the acetone excretion, as determined by this method, have little significance.

Folin's Method.

This method² yields only the preformed acetone occurring in the urine and does not regard the acetone which may be derived from the diacetic acid. If the urine be distilled and this method employed, the total possible acetone will result. It is somewhat more accurate than the previous method and is much more simple and less time-consuming. The writer finds it extremely serviceable and adopts it in all acetone determinations.

Twenty-five c.c. of urine are measured into an aerometer cylinder, similar to that used in Folin's ammonia apparatus, a few drops of 10 per cent. phosphoric acid, 10 grams of sodium chlorid, and a little petroleum are added. In the absorbing bottle which is fitted with an absorption bulb are placed 300 c.c. of water, 20 c.c. of 40 per cent. potassium hydrate, and an excess of tenth-normal iodine solution. The apparatus is connected with the filter-pump in the same manner as described under Ammonia and an air-current drawn through for one-half hour. The air should not be passed as rapidly as in the ammonia determination. Each worker should check his air blast by control estimations, using known solutions of acetone. In this way the acetone will be removed from the urine and converted, in the receiving flask, into iodoform. The contents of the receiving flask are acidified with concentrated HCl, using 10 c.c. of acid for every 10 c.c. of alkali previously used, and

¹ See Marriott, Jour. Biol. Chem., 1913, XVI, 281; Goodwin, Jour. Am. Chem. Soc., 1920, XLII, 39.

² Jour. Biol. Chem., 1907, III, 177; also, Folin and Denis, Ibid., 1914, XVIII, 263.

the excess of iodine titrated with tenth-normal sodium thiosulphate as in the previous method.

If this method be employed with the distilled urine, subtract from the total acetone, obtained in the distillate, the preformed acetone, derived from the fresh urine, and obtain the acetone referable to diacetic acid. If this value be multiplied by 1.758 the result will be the amount of diacetic acid, as such, present in the urine taken.

Shaffer has recently introduced a method for the determination of the total acetone, which is closely related to his method for estimating β -oxybutyric acid and will be discussed in a later section.¹

(b) **Diacetic acid** ($\text{CH}_3\text{—CO—CH}_2\text{—COOH}$).

This substance is derived from β -oxybutyric acid and is the precursor of acetone. As a rule, if acetone be present in large amounts, diacetic acid is also present and indicates a much graver condition than does the mere presence of acetone. If Folin's contentions are true, most of what we now call acetone is, in reality, diacetic acid, so that this latter substance should be tested for in routine urinary work. The remarks previously made regarding the appearance of acetone in the urine also hold for diacetic acid.² This substance is very volatile and disappears from the urine in a relatively short time so that the tests should be applied to perfectly fresh specimens.

Gerhardt's Test.

To 10 c.c. of urine are added a few drops of 10 per cent. ferric chlorid solution. This is best added as long as a precipitate of phosphates occurs, these latter bodies being then filtered off. To the filtrate are added a few drops more of ferric chlorid solution when the urine shows a Bordeaux-red color in the presence of diacetic acid. This color appears cherry-red by transmitted light and purplish-red by reflected light.

Unfortunately, however, this test is not specific for diacetic acid.³ A red color is observed in the presence of cyanates, formates, acetates, phenol compounds, salicylates, the conjugated glycuronates of phenacetin, antipyrin, thallin, kryofin, and kairin, as well as meconic acid which may be excreted after intake of opium. If the urine be heated, diacetic acid decomposes more or less completely into acetone and carbon dioxid, so that the red color due to diacetic acid will either disappear or become much weaker, while that with the other substances mentioned is not affected by heat. This same disappearance of color is noted if the mixture be allowed to stand from 24 to 48 hours. Schreiber recommends filtration of the urine through animal char-

¹ See Issoglio, *Giorn. farm. chim.*, 1919, LXVI, 301 and *Jour. Chem. Soc.*, 1919, CXVI, 304, for a nephelometric method.

² See Dakin and Dudley, *Jour. Biol. Chem.*, 1913, XVI, 515; Maillard, *Jour. pharm. chim.*, 1919, XX, 185.

³ See Steensma and Koopman, *Nederl. Tijdschr. v. Geneesk.*, 1914, LVIII, 800; Lichtwitz, *Berl. klin. Wchnschr.*, 1915, LII, 399; Barret, *Jour. Lab. and Clin. Med.*, 1917, II, 203; Mitchell (*Jour. pharm. chim.*, 1919, XX, 31) modifies this test by using ferric alum instead of chlorid. Maxwell (*Med. Jour. Australia*, 1920, I, 458) calls attention to the fact that urines of persons, who have been taking carbonates or bicarbonates, will show a red color when the ferric chlorid is added in the Gerhardt test. This is due to the interaction of the ferric chlorid with the carbonate or bicarbonate radical. While the color is not identical with that given by diacetic acid, yet it is confusing and may lead to errors of interpretation if the bicarbonates be not excluded.

coal, using about 10 grams of charcoal to 100 c.c. of urine. Antipyrin, phenacetin, and kryofin are retained while sufficient of the diacetic acid passes through to permit of detection. The urine may even be acidified with sulphuric acid and extracted with ether. This ethereal solution, which contains the diacetic acid, is then shaken with water and ferric chlorid solution added. A deep red color will be seen in the watery layer in the presence of diacetic acid. If the red color, on addition of ferric chlorid, be due to the presence of meconic acid, it will disappear on the further addition of stannous chlorid or of alkali hypochlorites, while that due to diacetic acid is unaffected. This test is, perhaps, more frequently used than any other test for diacetic acid in the urine, but is by no means as serviceable as the following.

Arnold's Test.

For the performance of this test two reagents are employed: (1) a solution consisting of 1 gram of paraamidoacetophenon, 100 c.c. of distilled water, and 2 c.c. of concentrated hydrochloric acid; (2) 1 per cent. sodium nitrite solution. Fifteen c.c. of urine are treated with a mixture consisting of 10 c.c. of solution 1 and 5 c.c. of solution 2 and one drop of concentrated ammonia added. In practically all urine, whether it contains diacetic-acid or not, a brownish-red coloration is observed which changes in the absence of diacetic acid into yellow if the mixture be treated with an excess of concentrated hydrochloric acid, while if diacetic acid be present the color changes to a beautiful purple on the addition of acid. If the mixture be shaken the foam also shows a distinct violet coloration.

As this test is somewhat difficult in the presence of small amounts of diacetic acid, *Lipliawsky*¹ has modified it as follows: Six c.c. of solution 1 and 3 c.c. of solution 2 are treated with the same volume of urine, a drop of ammonia is added and the mixture shaken, when it assumes a brick-red color. According to the probable contents of the urine in diacetic acid 10 drops to 2 c.c. of this mixture are treated with 15 to 20 c.c. of concentrated hydrochloric acid, 3 c.c. of chloroform and two to four drops of ferric chlorid solution. The test-tube is then closed with a cork and gently shaken for one-half to one minute. In the presence of traces of diacetic acid the chloroform assumes a characteristic violet coloration, while in the absence of this acid the color is yellow or light red. This test is positive for one part in 400,000 of water. Acetone and β -oxybutyric acid do not react with this test nor do the drugs previously mentioned under the Gerhardt Test interfere. If the urine is highly colored it is advisable to filter through animal charcoal.

(c) β -Oxybutyric Acid² ($\text{CH}_3\text{—CHOH—CH}_2\text{—COOH}$).

This acid, the mother substance of the acetone bodies, is found in the urine in extreme cases of the conditions described under Acetone. The amount excreted may vary from traces to as high as 100 grams. K  l  z has reported an excretion of 225 grams in 24 hours. The free acid is practically never found in the urine, being excreted either as the ammonium, sodium, or

¹ Deutsch. med. Wchnschr., 1901, XXVII, 151.

² The more scientific name for this body is *b*-hydroxybutyric acid. See Sassa, Biochem. Ztschr., 1914, LIX, 362; Kennaway, Biochem. Jour., 1914, VIII, 230 and 355.

potassium salt. These salts as well as the free acid are levorotatory and may be detected by the polariscope after previous fermentation of the carbohydrates.

In the condition known as diabetic coma a specific intoxication with β -oxybutyric acid is assumed as the causative factor. While this is undoubtedly true to a large extent it cannot be regarded as the only factor in diabetic coma, as administration of β -oxybutyric acid in large quantities will not necessarily lead to such a syndrome unless largely retained. We do undoubtedly have an acidosis which acts by ultimately depriving the tissues of fixed alkalies and may be regarded, therefore, as of great importance in following a diabetic case. Were this acid, *per se*, accountable for the entire symptomatology, we should be able, by administering alkalies, to overcome the effect of the acid intoxication. In some cases such therapy is extremely beneficial, while in others it is practically useless, as it cannot influence the formation of the toxic bodies and may not increase their elimination.

As previously stated, the ammonia output of the urine is an invaluable guide in following the course of an acidosis, especially in diabetes. One gram of ammonia (NH_3) is equivalent to 6.12 grams of β -oxybutyric acid, especially when in excess of the amount directly due to the food.

To detect β -oxybutyric acid follow the method of Hart.¹ To 20 c.c. of urine add 20 c.c. of water and a few drops of acetic acid and boil until the volume is reduced to about 10 c.c. (to remove acetone and diacetic acid). To the residue add sufficient water to make the volume 20 c.c. and place 10 c.c. in each of two test-tubes. To one of these add 1 c.c. of hydrogen peroxid, warm gently and allow to cool. Apply Lange's test for acetone (see p. 354) and allow the tubes to stand for a few hours. No reaction is observed in the tube which contains no hydrogen peroxid, while a distinct red zone is seen in the other.

Quantitative Determination.

A large number of methods have been advanced for the determination of β -oxybutyric acid in the urine. Among these we find the methods of Külz, Tollens, Wolpe, Magnus-Levy, Bergell, Stadelmann, and Darmstädter. While some of these are accurate under certain conditions, so many precautions must be taken that widely varying results may be obtained. On the other hand, the method of Magnus-Levy, which is undoubtedly very exact, requires 24 hours, while that of Bergell depends to a large extent upon the condition of the powder which is extracted with ether.

Black's Method.

This method² is a modification of those of Magnus-Levy and of Bergell, but in the writer's hands has given much more satisfactory results. One hundred c.c. of urine are faintly alkalinized with sodium carbonate and evaporated in a porcelain dish to one-third or one-fourth of the original volume. The residue is then concentrated to about 10 c.c. on a water bath in order

¹ Am. Jour. Med. Sc., 1909, CXXXVII, 869.

² Jour. Biol. Chem., 1908, V, 207. See Van Slyke (Jour. Biol. Chem., 1917, XXXII, 455) and Van Slyke and Fitz (Ibid., 495) for methods of determining the three acetone bodies. See, also, Lillig, Pharm. Ztg., 1910, LXIV, 606 and 707; Hubbard, Jour. Biol. Chem., 1921, XLIX, 351.

completely to remove the diacetic acid. This is then cooled, acidified with a few drops of hydrochloric acid, and made into a thick paste with plaster of Paris. This mixture soon begins to set, when it is stirred and broken up with a glass rod. This porous mass is then transferred to a Soxhlet apparatus and extracted with pure ether for two hours. The ether extract is evaporated, the residue taken up with water, decolorized with bone-black and filtered perfectly clear. The filtrate is then made up to 25 c.c. and its rotation determined with a polariscope. In determining the amount of β -oxybutyric acid from its rotation we must make use of the following calculation. The specific rotation of the free acid is -24.12 in a decimeter tube. One division of the scale in the case of glucose equals 2 per cent., so that we may find the percentage of β -oxybutyric acid by the following proportion:

$$2 : X :: 24.12 : 52.7. \quad X = 4.37 \text{ per cent.}$$

By a similar proportion it may be found that one division of the scale corresponds to 7.1 per cent. of sodium β -oxybutyrate, whose specific rotation is -14.35 .

This method is just as exact as those of Magnus-Levy and Bergell and has the advantages that it is simpler, more reliable, and may be performed in shorter time. The greatest difficulty with this method arises in the determination of the exact point of the scale at which the two portions of the polarimetric field are equally illuminated. Magnus-Levy has shown that a difference of $\frac{1}{10}$ of 1° in reading the polariscope amounts to about three grams per liter of β -oxybutyric acid, so that great care must be used with the polariscope in this method as well as in all others in which it is applied.

Shaffer's Method.

This method¹ seems to the writer to be the most desirable one that has been advanced. Shaffer and Marriott² show that this method yields uniformly about 90 per cent. of theoretical values. The results obtained must, therefore, be corrected by the addition of 10 per cent. of the amount found. See, also, Pribram³ in this connection.

The principle of the method is the oxidation of β -oxybutyric acid to acetone and carbon dioxide and the determination of the amount of acetone thus evolved. The acetone and diacetic acid already existing as such in the urine are previously determined by this method, so that it serves as a general one for the acetone bodies.

From 25 to 250 c.c. of urine, depending upon the amount of β -oxybutyric acid expected, are measured into a 500 c.c. volumetric flask and an excess of basic lead acetate and 10 c.c. of concentrated ammonia are added. In selecting the amount of urine to be taken one should use sufficient to yield from 25 to 50 mg. of acetone derived from β -oxybutyric acid. The solution in the flask is then diluted to the graduating mark, is thoroughly shaken, and filtered. Two hundred c.c. of the filtrate (representing $\frac{2}{5}$ of the original volume of

¹ Jour. Biol. Chem., 1908, V, 211.

² Jour. Biol. Chem., 1913, XVI, 265. Folin and Denis (Jour. Biol. Chem., 1914, XVIII, 263) show that their turbidity method, using small amounts of urine, yields theoretical amounts of acetone.

³ Ztschr. f. exp. Path. u. Therap., 1912, X, 279 and 284.

urine taken) are diluted with water to 500 or 600 c.c. Fifteen c.c. of concentrated sulphuric acid and a few grams of talcum are added, and the mixture distilled until 200 to 250 c.c. of distillate collects (distillate A).

In this distillation, the distilling flask, which may be an 800 c.c. Kjeldahl flask, should be fitted with a dropping funnel and water run in to prevent the volume of fluid in the flask from becoming less than 400 c.c. This distillate (A) contains the preformed acetone and that from the diacetic acid as well as volatile fatty acids which may be present in the urine. To remove the fatty acids, especially formic acid, the distillate A is redistilled after adding 5 c.c. of 10 per cent. sodium hydrate solution. This distillate (A_2) is then titrated with standard tenth-normal iodine and thiosulphate solutions as described in the Huppert-Messinger method for determination of acetone.

The residue of urine and sulphuric acid from which A was obtained is again distilled, dropping in 400 to 600 c.c. of 0.1 per cent. to 0.5 per cent. potassium bichromate¹ solution. This bichromate must not be added faster than the distillate collects unless the boiling liquid turns a pure green color, indicating that the bichromate is being used up more rapidly. When about 500 c.c. of distillate (B) have collected, 20 c.c. of 3 per cent. H_2O_2 are added to the distillate² together with a few c.c. of sodium hydrate solution and this redistilled. This second distillate (B_2) is then titrated with tenth-normal iodine and thiosulphate solution. One mg. of acetone represents 1.794 mg. of β -oxybutyric acid.

(4) Abnormal Pigments.

(a) Blood Pigments.

The principal blood pigment appearing in the urine is hemoglobin, which has been previously discussed under the heading of Protein in the Urine. Certain derivatives of this pigment are found, however, in conditions in which hemoglobin does not appear (see hemoglobinuria).

Hematoporphyrin.

This is an iron-free derivative of hemoglobin and appears to be present in minute traces in normal urine. Pathologically, it has been found in cases of rheumatism, phthisis, Addison's disease, pericarditis, paroxysmal hemoglobinuria, cirrhosis of the liver, exophthalmic goiter, croupous pneumonia, lead poisoning, syphilis, and many acute infectious diseases. Long-continued use of certain hypnotics, such as sulphonal,³ trional, and tetronal, is frequently associated with the appearance of hematoporphyrinuria.

¹ Cooke and Gorslin (Jour. Biol. Chem., 1911, X, 291) have wisely advised the following modification. The residue of urine and sulphuric acid is diluted to 600 c.c. and 5 c.c. of a 5 per cent. solution of potassium bichromate are added. Distill down to 300 c.c. and add water through the dropping tube to 500 c.c. Distill until 500 c.c. have been obtained. Additional 5 per cent. bichromate is run in, a few cubic centimeters at a time, whenever the distilling solution shows a greenish tinge.

² See Witzemann (Jour. Biol. Chem., 1918, XXXV, 83) for the effect of variations in available alkali on the yield of acetone in this process.

³ See Pförtner, Deutsch. med. Wchnschr., 1914, XL, 1563; Fischer, Ztschr. f. physiol. Chem., 1915, XCV, 34; Ibid., XCVI, 148; Schumm, Ibid., 183; Hoagland, Jour. Agric. Res., 1916, VII, 41; Rous, Jour. Exper. Med., 1918, XXVIII, 645; Schumm, Ztschr. f. physiol. Chem., 1919, CV, 158; Snapper, Nederl. Tijdschr. v. Geneesk., 1920, I, 1233; van Straaten, Ibid., 1920, II, 2530; Günther, Deutsch. Arch. f. klin. Med., 1920, CXXXIV, 257; Grynfeldt and Lafont, C. R. Acad. des Sc., 1921, CLXXXIII, 257.

Urine containing hematoporphyrin is usually dark-red in color, but may vary from a brownish-red or port-wine color to a distinct Bordeaux-red. Hammarsten has shown that this color is not entirely due to the hematoporphyrin, but partially to other abnormal pigments whose identity is not certain.

In examining urine for the presence of hematoporphyrin the spectroscopic method is practically the only one available. If this pigment be present in large amounts the urine may be directly examined with the spectroscope, showing the four bands of alkali hematoporphyrin discussed in the section on Blood. As this method is not always certain and not always easy of application, it would seem preferable to treat 50 c.c. of urine with 10 c.c. of 10 per cent. sodium hydrate solution. The precipitated phosphates carry down the pigments. This precipitate is then treated with 10 drops of concentrated hydrochloric acid and 15 c.c. of absolute alcohol. The solution is filtered if necessary and examined with the spectroscope when the two absorption bands of acid hematoporphyrin will be observed.

(b) Biliary Pigments.

Normally, bile pigments do not occur in the human urine. As was previously discussed in the section on Urobilin, biliary pigments may appear in the urine in conditions interfering with the passage of the bile into the intestine or when increased formation of biliary pigments from blood pigments has occurred and an associated obstruction of the bile-ducts is present.

We see, therefore, that choluria occurs in every case in which there is obstruction to the outflow of bile into the intestine. Thus in catarrhal jaundice, biliary calculi in the common duct, carcinoma of the liver, and cirrhosis, bilirubinuria is frequent. Moreover, we may also find biliary pigments arising from purely hematogenous conditions, such as pernicious anemia, malaria, typhoid fever, arsenical poisoning, and yellow fever. Whether this latter type is not really hepatogenous in origin, as Stadelmann believes, is still unsettled, but it would seem more plausible to assume a primary breaking down of the red cells and a secondary insufficiency of the liver.

The chief biliary pigment found in the urine is bilirubin,¹ which is intermediate between hemoglobin and urobilin. On oxidation of this pigment, either in the system or in the methods of examination, various other pigments may arise. Thus we may find biliverdin, bilicyanin, bilifuscin, biliprasin, cholecyanin, and choletelin. In the fresh urine bilirubin is the only pigment noted.

A bile-containing urine may show various shadings of color, ranging from a greenish-yellow, through yellowish-brown, deep brown, or greenish-brown, to a pure green. If the urine be shaken, a yellowish or greenish-yellow foam is observed, while in normal urines the foam is practically colorless. The

¹ See van den Bergh and Snapper, *Nederl. Tijdschr. v. Geneesk.*, 1915, II, 469; Küster, *Ztschr. f. physiol. Chem.*, 1915, XCIV, 136; Hooper and Whipple, *Jour. Exper. Med.*, 1916, XXIII, 137; *Am. Jour. Physiol.*, 1916, XL, 332; Whipple and Hooper, *Ibid.*, 349; Garnier and Magnenand, *C. R. soc. biol. de Paris*, 1916, LXXIX, 278; DeJager, *Nederl. Tijdschr. v. Geneesk.*, 1916, II, 227; *Pharm. Zentralbl.*, 1917, LVIII, 442; Schneider, *Jour. Am. Med. Assoc.*, 1920, LXXIV, 1759; Küster, *Ztschr. f. angew. Chem.*, 1921, XXXIV, 246; Canelli, *Pediatrics*, 1921, XXIX, 495; Meulengracht, *Deutsch. Arch. f. klin. Med.*, 1921, CXXXVII, 38; Haessler, Rous and Brown, *Jour. Exp. Med.*, 1922, XXXV, 533.

presence of an excess of urobilin may also give a brownish foam. Urine containing bile always shows the presence of nuclealbumin along with slight traces of serum albumin, so that bile should be regarded as a source of extraneous albumin. The sediment will usually be more or less colored by the biliary pigment, the casts in yellow fever, for instance, being usually distinctly bile-stained.

Qualitative Tests.

A large number of tests have been advanced for the detection of biliary pigments in the urine and many of them are distinctly unsatisfactory. If large amounts of bile be present, the urine, acidified with HCl, may be shaken out with chloroform which dissolves the bilirubin. If the chloroform be evaporated, rhombic crystals with rounded edges or distinct needles of a brownish-red color will be observed. These crystals will give the color tests mentioned below.

Smith's Test.

This test has been described under other names, as those of Trousseau, Kathrein, Rosin, and Marechal. A few c.c. of urine, acidified if necessary with acetic acid, are treated with a 1 per cent. alcoholic solution of iodine in such a way that the latter solution is superimposed upon the urine, forming a distinct line of contact. If bilirubin or other biliary pigments be present, a beautiful emerald-green color is observed at the point of contact. This test is especially recommended, but it is not very sensitive, indicating only one part of biliary pigment in 10,000 of urine.¹ Certain drugs, especially antipyrin, may lead to the formation of a green color with this test. Thymol, if used as a preservative, may give rise to confusion with this and other bile tests.

Gmelin's Test.

One or two c.c. of nitric acid are placed in a test-tube and the same amount of urine is allowed to flow from a pipet in such a way that a distinct contact line is formed. If the nitric acid contains a trace of nitrous acid and the urine biliary pigment, a distinct green ring will be observed at the line of contact. In some cases, as the nitric acid oxidizes the pigment, a play of colors may be seen from green, through a blue, violet, and red, to a yellow. The primary green is the characteristic color, other colorations being occasionally due to pigments other than biliary. This test is supposed to indicate 1 part of bilirubin in 80,000 of urine.

If the urine contains an excess of indican a deep-blue ring may be observed or the combination of this blue with the yellow of the urine may give a green. In the presence of skatoxyl a violet-red ring may be noted, while various medicaments may give colorations ranging through the entire spectrum. In such cases it is always advisable to extract the acidified urine with chloroform and apply the test either to the evaporated residue or to its aqueous solution.

This test is, perhaps, more frequently used than any of the biliary tests, but requires considerable experience for its proper interpretation. If the urine be diluted the test is somewhat more distinctive. Many modifications of this test have been advocated, the most serviceable being the following:

¹ See Silberstern, *Zentralbl. f. inn. Med.*, 1922, XLIII, 185.

Rosenbach's Test.

A large quantity of urine, which has been acidified with HCl, is filtered several times through a thick filter-paper, which will hold back the bile-stained elements of the urine. It is sometimes advisable to add a little milk of lime to the urine before filtering, instead of the HCl, as this will throw down the phosphates which will carry with them the biliary pigment. If the filter-paper and contents be dried by pressing with a second dry filter-paper and a drop of yellow nitric acid allowed to fall upon it, distinct rings will be seen, which will be colored as in the previous test, the green one being external.

Nakayama's Test.

This is a modification of the older Huppert test.¹ Five c.c. of acid urine are treated with an equal volume of 10 per cent. barium chlorid solution and the mixture centrifuged. The barium chlorid precipitates the phosphates and sulphates and carries down the biliary pigments. The supernatant fluid is then poured off and 2 c.c. of the following reagent are added to the precipitate. The reagent consists of 99 c.c. of 95 per cent. alcohol, 1 c.c. of concentrated HCl, and 0.4 gram of ferric chlorid. If this mixture of precipitate and reagent be heated to boiling, a bluish-green or a brilliant green solution is obtained, which becomes violet or red on the addition of nitric acid. This test is said to indicate one part of bilirubin in 1,200,000 parts of urine.

Hammarsten's Test.

As the reagent in this test we use a mixture of one part of 25 per cent. HNO₃ and 19 parts of 25 per cent. HCl. Before use one part of this reagent is mixed with four parts of alcohol. A few drops of the urine are added to this mixture, when it assumes a green color in the presence of biliary pigments. If the urine be treated as in Nakayama's test and the precipitate mixed with 1 to 2 c.c. of the acid alcohol reagent and the whole centrifuged for a short time, a green solution is obtained if one part of bilirubin in 1,000,000 of urine be present.

Bile Acids.

The bile acids, taurocholic and glycocholic acids, are found in the urine in the form of their sodium salts. They may be found in small amounts in the same conditions in which the biliary pigments are present, but their amount is usually so small that they cannot be detected, as a rule, without being previously isolated. It seems to be fairly well established that the bile acids must be present to the extent of $\frac{1}{2}$ per cent. before detection in the urine is possible.

As the clinical significance of these acids is the same as that of the pigment and as their amount is so small that the methods of isolation require a fairly large volume of urine, the writer will refer to works on physiologic chemistry for such procedures.²

No absolutely reliable test is known for the detection of the bile acids in the

¹ See Rosenbloom, *New York Med. Jour.*, 1914, XCIX, 220.

² See Wieland and Sorge, *Ztschr. f. physiol. Chem.*, 1916, XCVII, 1; *Jour. Chem. Soc.*, 1916, CXL, 710; Foster and Hooper, *Jour. Biol. Chem.*, 1919, XXXVIII, 355, 367, 379, 393, 413 and 421.

untreated urine. There are, however, a few tests which are occasionally given providing the bile acids be present in sufficient amount.

Hay's Test.

This test depends upon the reduction of the surface tension of the urine in the presence of the bile acids. As advocated by Beddard and Pembrey, a pinch of powdered sulphur is sprinkled upon the surface of urine which should be preferably at a temperature not over 17°C. In normal urines the sulphur will float upon the surface, while if the urine contains bile acids the sulphur may sink at once indicating one part in 10,000 or may sink only after a few seconds to one minute, in this latter case indicating one part in 50,000. According to Sahli this test does not discriminate between biliary acids and biliary pigments, but clinically it is a matter of indifference which one is present. Phenol or aniline compounds lower the surface tension of the urine so that their presence may lead to wrong conclusions.¹

Oliver's Test.

This test is based upon the well-known property possessed by the bile acids of precipitating peptone when in acid solution. The reagent is as follows:

Powdered peptone,	8.33 grams
Salicylic acid,	1.12 grams
Acetic acid,	2 drops
Distilled water,	1 liter

One to two c.c. of clear filtered urine are placed in a test-tube and treated with 5 c.c. of the reagent. In the presence of bile acids a decided milkiness appears at once, being the more intense the larger the amount of bile acids.

Other tests, such as those of Pettenkofer and Udránzky, are only serviceable in the testing of the isolated bile acids.

(c) Melanin.

In cases of melanotic tumors the urine not infrequently contains a chromogen (melanogen), which is converted into melanin on allowing the urine to stand or after adding alkalies or oxidizing agents. The urine when freshly voided is normal in color, but on exposure to air gradually darkens until it becomes distinctly black. This coloration is first noticed in the upper portion of the urine and gradually extends toward the bottom. If ferric chlorid be added to the urine the coloration may be somewhat intensified so that one may assume an excess of phenol derivatives. It is to be remembered that indican must first be split up with acid before giving the coloration with ferric chlorid; moreover, melanin is insoluble in chloroform while the indigo is readily soluble. (See Eppinger.²)

The addition of the ferric chlorid may produce a black precipitate which is soluble in sodium carbonate solution, from which it may be reprecipitated

¹ See Allen (*Jour. Biol. Chem.*, 1915, XXII, 505) for a method of measuring accurately the variation in surface tension of the urine. See Müller, *Schweiz. med. Wehnschr.*, 1921, LI, 821; *Ibid.*, 1922, LII, 110.

² *Biochem. Ztschr.*, 1910, XXVIII, 181

by mineral acids. This pigment may also be found in some cases of chronic malaria¹ so that it is not absolutely pathognomonic of melanotic tumors.

(d) Phenol Derivatives.

As previously mentioned in the discussion of the variations in color of the urine, it may be dark colored either on voiding or after standing. While such colorations may be due to melanin, they are much more frequently traceable to the presence of sulphuric acid in conjugation with phenol,² paracresol, pyrocatechin, and hydroquinone. These substances are excreted especially in conditions associated either with increased intestinal putrefaction or with putrefactive processes elsewhere in the system. Aside from direct poisoning with these substances, they may be regarded as having much the same clinical significance as indican.

Qualitative and quantitative tests for these substances are rarely of clinical importance. They may be roughly determined by estimating the amount of ethereal sulphates in the urine, when a large increase may be assumed to be referable to these bodies unless indican is greatly in excess.

(e) Alkapton.

In certain conditions of disturbed protein metabolism, the urine contains pigments which cause it to turn dark on the addition of alkali or on standing. These urines are normal in color when voided and become black almost immediately on the addition of alkali, whence the name alkapton bodies and *alkaptonuria* for their excretion in the urine. The substances causing this change of color are hydroquinon-acetic acid (as it has been prepared synthetically from gentisic aldehyd, it has been called *homogentisic acid* with the formula $C_6H_3(OH)_2(CH_2COOH)$ and uroleucic acid, whose structure has not been absolutely settled.³ The former of these acids is present in all cases of alkaptonuria, while the latter may be present in all cases, but in such small amounts that it remains undetected. Urines containing these bodies strongly reduce copper and ammoniacal silver solutions, while bismuth solutions are

¹ Urriola (Interstate Med. Jour., 1912, XIX, 74) claims that a urinary excretion of blood pigment (probably hematin and not melanin) is invariably present as a pathognomonic sign of malaria. This may appear in the centrifuged specimen as a very abundant intense black pigment; a blue pigment, very constant but small in amount; or as an ochre pigment occasionally. See Herzog and Zeller, Biochem. Ztschr., 1919, XCVI, 233; Saccardi, Gazz. chim. ital., 1919, XLIX, (1), 201; Ibid., 1920, L, (1), 222; Ibid., 1920, L, (11), 118; von Hoeft, Biochem. Ztschr., 1920, CIV, 1; Rondoni, Sperimentale, 1920, LXXXIV, 157; Brahn and Schmidtman, Arch. path. Anat., 1920, CCXX, 137; Salkowski, Ibid., 1920, CCXXVIII, 468.

² See Hensel, Ztschr. f. physiol. Chem., 1912, LXXVIII, 373; also, Folín and Denis, Jour. Biol. Chem., 1915, XXII, 305 and 309; Siegfried and Zimmermann, Biochem. Ztschr. 1915, LXX, 124; Dubin, Jour. Biol. Chem., 1916, XXVI, 69; Anderson, Ibid., 387, 401 and 409; Moore, Am. Jour. Dis. Child., 1917, XIII, 15; Dubin, Jour. Biol. Chem., 1917, XXXI, 255; Tsudji, Ibid., 1919, XXXVIII, 13. For studies of phenols in blood see Benedict and Theis, Jour. Biol. Chem., 1918, XXXVI, 95 and 99; Weiss, Biochem. Ztschr., 1920, CX, 258; Simpson, Jour. Am. Med. Assoc., 1920, LXXV, 1204; Tisdall, Jour. Biol. Chem., 1920, XLIV, 409; Chaplin, Ibid., 1921, XLVII, 309; Bell, Jour. Inf. Dis., 1921, XXIX, 424; Sullivan and Dawson, Arch. Int. Med., 1921, XXVIII, 166; Pelkan, Jour. Biol. Chem., 1922, L, 491, 499, and 513.

³ See Mörner, Ztschr. f. physiol. Chem., 1912, LXXVIII, 306; Oswald (Ztschr. f. physiol. Chem., 1914, XCIII, 307) believes this acid is identical with homogentisic acid. See, also, Gross, Biochem. Ztschr., 1914, LXI, 165.

little affected. The so-called *glycosuric acid* of Marshall is probably identical with homogentisic acid and is not a definite chemical entity.

This condition is observed at various periods of life and seems to be of theoretical more than practical interest, as of the 78 cases reported none appeared to be much disturbed in health, the condition usually being detected accidentally.¹

The source of these substances is still uncertain. Baumann and Wolkow believe that the influence of specific bacteria in the intestinal canal upon the tyrosin, formed in the hydrolytic cleavage of the proteins, leads to direct formation of homogentisic acid. While this accounts for some of the abnormal substance, it cannot account for the entire amount of 3 to 7 grams of homogentisic acid excreted in the 24 hours. From the work of Meyer, Falta, Langstein and Wohlgemuth it would appear that this condition depends not on an abnormal formation of homogentisic acid but on an incapacity for further oxidizing it when formed. Dakin² believes that both factors play a part. However this may be, alkaptonuria has very few ill-effects, although transitory symptoms have been observed in occasional cases of diabetes, cirrhosis of the liver, tuberculosis, pyonephrosis and gastritis. The browning of the cartilage occurring in ochronosis is supposed by Albrecht and Zdarek to have some relation to the alkapton bodies, although Langstein could not show the alkapton acids in the urine in such cases.

Qualitative and quantitative tests for these acids are rarely necessary.³ The characteristic change of color on alkalinizing the urine is, at least, suggestive of homogentisic acid. Like all other hydroxy derivatives of benzol, this substance shows the Schiff reaction with ferric chlorid, producing a transitory blue coloration when present in amounts of 1 to 4,000. The reduction of copper solutions should not be mistaken for sugar, as homogentisic acid neither ferments nor shows any optical activity. The urine will usually show a relatively high acidity. As this substance is practically harmless in the system we should expect to find, as we actually do, no increase in the conjugated sulphates or glycuronates nor any increase in the ammonia content of the urine (Neuberg).

(f) Ehrlich's Diazo Reaction.

Under certain pathologic conditions the urine has been found to contain a chromogen which gives a deep red color to the urine when treated with

¹ See Poulsen, Münch. med. Wchnschr., 1912, LIX, 364; also, Baldwin, Am. Jour. Med. Sc., 1913, CXLV, 123.

² Jour. Biol. Chem., 1911, IX, 151. See, also, Pincussohn, Ergeb. d. inn. Med., u. d. Kinderheilkde., 1912, VII, 454; Süderbergh, Nord. Med. Arch., 1915, XLVIII, 1; Scheltema, Nederl. Tijdschr. v. Geneesk., 1915, II, 2050; Maggiore, Pediatria, 1916, XXIV, 234; Katsch, Deutsche Arch. f. klin. med., 1918, CXXVII, 210; Schochet, Arch. Int. Med., 1918, XXII, 82; Gross, Deutsch. Arch. f. klin. Med., 1919, CXXVIII, 249; Katsch, Ibid., 1920, CXXXIV, 59; Widmark, Hygiea, 1920, LXXXII, 309; Debenedetti, Policlinico, 1920, XXVII, 1379; Rombach, Nederl. Tijdschr. v. Geneesk., 1921, I, 1240; Katsch and Nemet, Biochem. Ztschr., 1921, CXX, 212; Gibson and Howard, Arch. Int. Med., 1921, XXVIII, 632; Bilderback, 1922, XXIII, 259; Oppenheimer, Arch. Int. Med., 1922, XXIX, 732.

³ Briggs (Jour. Biol. Chem., 1922, LI, Am. Jour. Dis. Child., 453) has introduced a colorimetric method for the determination of homogentisic acid.

diazo compounds and ammonia. This is known as the diazo reaction, the urinary substance causing it being somewhat uncertain. According to Bondzynski, the alloxypoteic acid is the causative factor, but this needs confirmation, as Clemens has apparently shown that the body producing the reaction is sulphur-free.

In the performance of this test two reagents are used: (1) sulphanilic acid 5 grams, 50 c.c. of concentrated hydrochloric acid, and 1,000 c.c. of water; (2) $\frac{1}{2}$ per cent. aqueous sodium nitrite solution.

To a mixture consisting of 50 parts of solution 1 and one part of solution 2 is added an equal volume of the urine. The mixture is then shaken and about one-tenth of the bulk of ammonia added quickly, and the mixture thoroughly shaken. The ammonia may be added in such a way that a line of contact forms, this latter method frequently bringing out a much more beautiful reaction. According to Greene, a mixture consisting of 100 parts of solution 1 to one of solution 2 renders the test more delicate. Instead of sulphanilic acid, paraamidoacetophenon may be used, as suggested by Friedenwald.

If the test be positive the entire urine will assume an intense red coloration or a colored ring will be observed at the point of contact between the ammonia and the mixture. With normal urine a distinct orange color may be observed. On shaking the mixture the foam will be more or less brilliant red in color, which is more characteristic than the red coloration of the mixture. On allowing the mixture to stand for 24 hours a green precipitate may be observed at the bottom of the tube, which Ehrlich regards as especially characteristic of the true diazo reaction. This green precipitate is not always present and is not necessary for a positive reaction, the red coloration of the mixture and of the foam being more frequent.

The administration of certain drugs may markedly affect this test.¹ Thus we find that naphthalin, chrysarobin, opium, and phenol derivatives give a reaction which is very similar to the true diazo reaction, but may be distinguished, according to Wood, by the fact that color is more permanent in alkaline solutions, does not fade to any extent on the addition of a strong mineral acid, the foam is more yellow than in the typhoid reaction, and the green precipitate does not appear on standing. Burghart has found that, following the administration of tannic acid, gallic acid, tannigen, and tannalbin, the diazo reaction disappears from the urine, the inhibiting effect being perhaps exerted upon the reagents used rather than upon the unknown factor which usually causes the coloration.

According to Ehrlich, if the urine contains an excess of biliary pigment, a dark cloudy discoloration may occur, which is changed on boiling to a distinct reddish violet. He also finds on applying the diazo test in some cases that the urine and foam become yellow before the addition of ammonia. After ammonia is added the color changes to a lighter yellow. This reaction is known as Ehrlich's "*egg-yellow*" reaction, and is supposed to be due to the presence of urobilinogen. He regards this latter test as especially important in predicting the crisis of pneumonia.

¹ Skorczewski und Sohn (Wien. klin. Wchnschr., 1911, XXIV, 1700) show that the urine of patients taking atophan gives this reaction.

This diazo reaction is never positive in health. It was formerly regarded as pathognomonic of typhoid fever, but it has been shown to occur in many other conditions. It is true that in typhoid fever it may be present as early as the third or fourth day and may persist for some time, reappearing if a relapse occurs, while the Widal test, as will be remembered, does not vary with a relapse. Moreover, the intensity of this reaction is somewhat parallel with the severity of the case, while the Widal reaction may not be present in the severest types of typhoid fever. A positive diazo reaction occurs frequently in measles, somewhat less frequently in pneumonia, miliary tuberculosis, scarlet fever,¹ diphtheria, and erysipelas, while in rheumatism and meningitis it is rarely obtained. Michaelis believes that the presence of a positive diazo reaction in pulmonary tuberculosis indicates a progressive condition with a grave prognosis.²

This reaction is, therefore, a valuable aid in diagnostic work. As it is present in about 80 per cent. of cases of typhoid fever, a negative reaction would not necessarily exclude typhoid nor would a positive reaction prove its presence. Its appearance in a relapse is of value, as the Widal test would give no information under such conditions. It is to be said, however, that the diagnosis should by no means rest upon this test.

(g) Russo's Test.

This test³ has been recently advanced and seems to have somewhat more diagnostic importance than has the diazo reaction. The technic is very simple and is as follows: Four drops of a 1 to 1,000 aqueous solution of methylene blue are added to 4 or 5 c.c. of suspected urine. If the reaction be positive the mixture turns to an emerald or mint-green hue. A light-green or bluish-green tint shows a negative reaction. The positive reaction is not affected by boiling the urine or by the previous ingestion of such compounds as phenacetin, salol, quinin, and calomel. The difficulty in the application of the test comes in the ability to recognize the various tints of green which may be present. With a little practice, however, a positive reaction may be readily detected, especially if a control test be made with normal urine.

This test is shown as early as the second day of typhoid fever and persists throughout its course. The mint-green hue is first observed, the emerald-green tint appearing as the disease progresses. If the course is favorable the color tone becomes more and more bluish, while if unfavorable the emerald tint persists. This test is also given in measles, smallpox, chronic

¹ See Ueber, *Med. Klin.*, 1912, VIII, 322; Woody and Kolmer, *Arch. Pediat.*, 1913, XXIX, 18; Marcantoni, *Gazz. d. osp.*, 1914, XXXV, 193; *Jahrb. f. Kinderh.*, 1915, LXXXI, 168.

² See Heflebower, *Am. Jour. Med. Sc.*, 1912, CXLIII, 221; Pick, *Med. klin.*, 1915, XI, 1292; Levy, *Deutsche med. Wehnschr.*, 1915, XLI, 1212; Bosch, *Ibid.*, 1916, XLII, 17; Sinclair, *Jour. A. M. A.*, 1916, LXVI, 247; Corper and Callahan, *Jour. Lab. and Clin. Med.*, 1916, 1, 740; Pottenger, *Ibid.*, II, 37; Masoim, *Bull. acad. roy. med. Belg.*, 1919, XXIX, 982; Guth, *Beitr. z. klin. Tuberk.*, 1920, XLV, 108; Hermanns and Sachs, *Ztschr. f. physiol. Chem.*, 1921, CXIV, 79; Wahlberg, *Finiska Läkare, Handl.*, 1921, LXIII, 360; Kilduffe, *Medical Record*, 1922, CI, 500.

³ *Riforma Medica*, 1905, XXI, 507; abstract *Jour. Am. Med. Assn.*, 1905, XLV, 363; Kahn and Wechsler, *Med. Record*, 1916, LXXXIX, 106; Boit, *Beitr. z. klin. d. Tuberk.*, 1918, XXXVIII, 154; Skutetzky and Klastan, *Wien. klin. Wehnschr.*, 1918, XXXI, 1016.

and suppurative tuberculosis, but is negative in varioloid, varicella, scarlet fever, miliary tuberculosis, appendicitis, and malaria.

This test is as simple and just as reliable as is the diazo-reaction, being especially valuable in differentiating a typhoid from a miliary tuberculosis. Rolph and Nelson¹ have pointed out that urines containing bilirubin react to this test, so that this fallacy must be borne in mind.

(h) Dimethylaminobenzaldehyd Reaction.

This test, advanced by Ehrlich, is as follows: Prepare a 2 per cent. solution of p-dimethylaminobenzaldehyd in equal parts of concentrated HCl and water. Add a few drops of this solution to 5 c.c. of fresh cold urine and allow to stand for a few minutes. A positive reaction is indicated by the appearance of a cherry-red color, which may be extracted with chloroform or epichlorhydrin. Heating facilitates the reaction, but here normal urine may give a slight reddish coloration. In the cold normal urine gives a greenish-yellow color. Apparently this coloration is due to the presence of metabolic products derived from blood pigments (urobilinogen compounds, see p. 284).² It is to be expected, therefore, that this reaction would be distinct in diseases of the liver and bile passages, although it is not constant even here. It is, also, observed in tuberculosis, pneumonia, typhoid fever, malaria and scarlet fever.³ It has little clinical value.



FIG. 89.—Purdy electric centrifuge.

IV. MICROSCOPIC EXAMINATION OF THE URINE.

The microscopic examination of the urine is important in every case. So varied are the elements which appear in the microscopic field that considerable experience is necessary before absolute interpretation can be made. Not infrequently the character of the sediment will change a diagnosis, as,

¹ Medical Record, 1911, LXXX, 373. See, also, Neuman and Behrend, Arch. Int. Med., 1913, XI, 456; da Pozzo, Gazz. d. osp., 1914, XXXV, 865; Petzetakis (Lyon Méd., 1916, CXXV, 309) reports a similar test performed by the addition of two or three drops of a 5 per cent alcoholic solution of iodine to 10 to 15 c.c. of urine. A golden yellow tint is a positive reaction, which he claims is shown in "open" tuberculosis and typhoid fever, but not in "closed" tuberculosis. See, Ruiz and Moliner, Arch. Esp. de Pediat., 1919, III, 420.

² Fischer and Meyer-Betz (Ztschr. f. physiol. Chem., 1911, LXXV, 232) show that the reaction depends on the presence of a dipyrrolylphenylmethane pigment of the class of non-stable pyrrol derivatives, to which group the blood and biliary pigments, as well as urobilinogen, belong.

³ See Hesse, Med. Klin., 1913, IX, 294; also, Steensma, Nederl. Tijdschr. v. Geneesk., 1914, LVIII, 467; Rabinowitsch, Berl. klin. Wchnschr., 1914, LI, 1456; Berkowitz, Med. Record, 1914, LXXXVI, 1087; Eisner, Deutsch. Ztschr. f. Chir., 1915, CXXXII, 589; Robertson, Calif. State Jour. Med., 1915, XIII, 65; Litzenberg (Chicago Gynec. Soc., Apr. 16, 1915) reports an increase of urobilinogen in the toxemias of pregnancy.

for instance, when pus- or blood-cells are present in sufficiently large amounts to account for an albuminuria previously determined by chemical methods. It is, therefore, essential that the microscopic examination of urine form a part of the ordinary routine.

In obtaining the sediment of the urine for microscopic examination, two methods are possible. In the first place, the urine may be allowed to stand in a conical glass for periods ranging from 12 to 24 hours. The sediment originally present in the urine as well as that formed by chemical changes taking place during the standing will collect in the lowest portion of the glass and may be removed by a glass-tube drawn out to a somewhat small point. It is to be remembered that a sediment at the end of 12 to 24 hours may be entirely different from that originally present in the freshly voided specimen. The changes in the reaction of the urine will, necessarily, lead to the dissolving of certain types of crystals and to the formation of other varieties. Moreover, casts, if originally present, may dissolve or disappear as a result of the reaction of the urine becoming alkaline. For these reasons it is absolutely essential, in the use of this gravity method, that preservatives be added to the urine. Among the preservatives which may be used to prevent bacterial action during the sedimentation, we find a small piece of camphor or a rather large crystal of thymol serving the purpose. Some workers add one-fifth the volume of a 4 per cent. solution of borax which is equally



FIG. 90.—
Sediment tube
for Purdy cen-
trifuge.

useful, but the addition of chloroform or formalin, does not serve as well in these cases, as the former does not completely preserve the casts and the latter introduces a crystalline compound of formalin and urea which may be confusing, as it is not unlike impure leucin.

For the detection of casts and other sediments in the urine, when these are sparsely present, the method of Haines and Skinner¹ may, often, be advantageously used. A considerable volume of urine, usually 250 to 500 c.c., is placed in a conical (Oldberg) percolator, a few grams of hydrated chloral in solution are added to retard decomposition, and the specimen set aside in a cool place for 12 to 24 hours. At the end of this time the lowermost stratum of urine is drawn off and centrifuged. The deposit thus obtained represents practically all the casts in the volume of urine employed. This method often reveals casts when the usual centrifuge method fails to show them and, with it, very



FIG. 91.—Per-
centage centri-
fuge tube.

few specimens, even normal ones, are found which do not show casts.

Secondly, the sediment may be thrown down by the use of the centrifuge. This apparatus is seen in the accompanying cut. By the use of this method no preservative is needed, a deposit is obtained within three minutes in a

¹ Jour. Am. Med. Assoc., 1898 XXX, 234.

much more concentrated form, and changes in the sediment do not take place. The writer would recommend, therefore, the use preferably of the electric centrifuge or, at least, of the type run by hand.

Whatever method may be adopted for obtaining the urinary sediment, the next steps in the process are the same. A pipet, consisting of a glass tube drawn out to a point about one-half the diameter of the tube, is introduced to the bottom of the vessel containing the sediment, a finger being placed over the upper end to prevent fluid passing into the tube as it is introduced. When the tip of the pipet comes in contact with the deposit, the pressure of the finger on the upper end of the pipet is removed and the deposit allowed to flow up into the pipet. The finger is then placed tightly over the tube as it is withdrawn from the fluid. By placing the tip of the pipet in contact with a perfectly clean slide which is absolutely free from scratches and by gradually rotating the pipet, a small portion of the sediment is obtained. A cover-glass is then placed upon this drop avoiding any undue pressure which might distort the organized elements of the sediment. Some workers dispense with the coverglass and use somewhat larger amounts of sediment, but the writer does not find this method as acceptable owing to the fact that the lens of the objective may dip into the fluid and thus give indefinite microscopic pictures. Moreover, the focus cannot be as accurately adjusted without the use of the cover-glass.

In the examination of the microscopic specimen, prepared as above, the point of special importance to be observed is the proper adjustment of the light. The writer is accustomed to use the low-power objective in the preliminary examination. In this case it is essential that the light be shut off to a large extent, as the recognition of casts cannot be made in a brilliantly illuminated field. This examination with the low power has the advantages that larger visual fields are subject to inspection, casts are easily recognized and crystalline deposits, as well as morphological elements, are usually differentiated. After the preliminary examination with the low power, the final examination is made with the high-power dry lens. In this way elements which appear suspicious under the low power are more clearly brought out and differentiations made possible between various types of cellular elements. With the high-power lens it is, of course, essential that the field be somewhat more illuminated than when the low-power is used. While a mechanical stage is, at times, advantageous in the microscopic examination of the urine, the writer has found that the fingers serve practically every purpose in the manipulation of the slide under examination.

Where the urine is to be examined for the presence of bacteria, stained specimens must be made and examined with the oil-immersion lens.

Urinary sediments are classified into two divisions: (a) chemical or nonorganized and (b) anatomical or organized sediments. The nonorganized sediments exist in solution in normal urine and appear as deposits under conditions of excessive formation, excessive excretion, or of alterations in the urine affecting its solvent properties. The chief chemical sediments are uric acid and its salts, calcium oxalate, phosphates, sulphates, cystin, leucin,

tyrosin, xanthin, fat, and fatty acids. The organized sediments are usually foreign substances and are not met with in normal urine. They consist of epithelial cells, pus corpuscles, blood-cells, renal casts, spermatozoa, infusoria, bacteria, and tissue fragments.

(A) **Unorganized Sediments.**

(a) **Those Appearing in Acid Urine.**

(1) **Uric Acid ($C_5H_4N_4O_3$).**

This occurs as a sediment in the urine under 3 conditions: (1) great concentration, (2) high acidity, and (3) low temperature. The deposit differs from others in possessing a deep yellow or orange-red color, although some of the smaller crystals are occasionally colorless. The primary form of the uric acid crystal is that of the rhombic prism. Modifications of this, in the

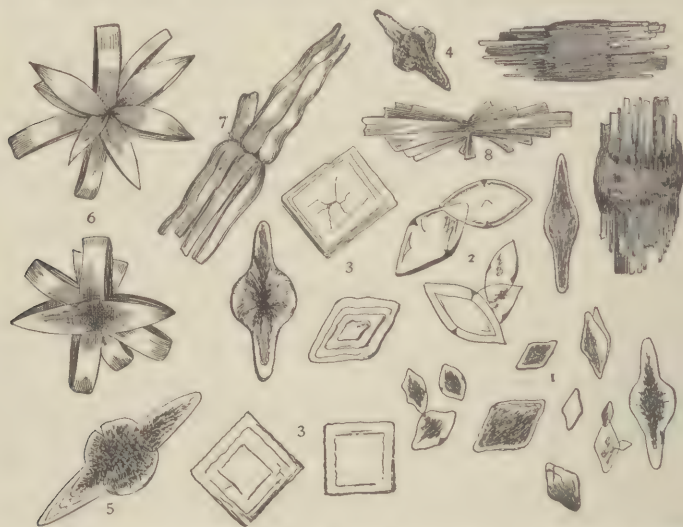


FIG. 92.—Various forms of uric acid. 1, Rhombic plates; 2, whetstone forms; 3, quadrate forms; 4, 5, prolonged into points; 6, 8, rosettes; 7, pointed bundles; 9, barrel forms precipitated by adding hydrochloric acid to urine. (Hawk.)

form of square plates, cubes, ovoids, dumb-bells, or whetstone crystals are sometimes noticed. A rare type, especially of the colorless crystals, is a perfect hexagon which resembles cystin so closely that chemical means of differentiation must be used. The crystals may be single or grouped in rosettes or fan-shaped masses. Occasionally typical needle-shaped crystals may be seen which are arranged in sheaves.

The microscopic peculiarities of uric acid are usually such as to permit of its easy recognition. In some cases, however, it is wise to confirm the microscopic finding by the *murexid* test as follows: Place a small quantity of the sediment in an evaporating dish and add a few drops of concentrated nitric acid. Evaporate on the water-bath to dryness, when a yellowish or reddish residue will remain. Allow the residue to cool and add a few drops of ammonium hydrate solution. In the presence of uric acid a distinct reddish purple color will appear. If water be added to this purple solution and the mixture evaporated to dryness the color disappears. This latter point is

of importance as xanthin, which may resemble unusual types of uric acid in microscopic appearance, also gives the murexid test, but the color does not disappear on heating with water.¹

(2) **Sodium Acid Urate** ($C_5H_3NaN_4O_3$).

This salt of uric acid forms the bulk of the "brick-dust deposit" or "sedimentum lateritium" found when urine has cooled. In such cases the urine first shows a milky appearance and the sediment soon settles on the sides and bottom of the container. This deposit is usually in the form of irregular



FIG. 93.—Acid sodium urate. (*Hawk.*)

amorphous granules of a brownish or pink color. Occasionally the sediment may be distinctly crystalline, occurring as prismatic needle-like crystals which are grouped in star-shaped, fan-shaped or dumb-bell-like clusters.



FIG. 94.—Xanthin. (*Hawk.*)

(3) **Potassium Acid Urate** ($C_5H_3KN_4O_3$).

This substance occurs only as a granular amorphous deposit. Owing to its greater solubility, it does not form as large an amount of the brick-dust deposit as does the sodium salt.

¹ See Kohler, *Ztschr. f. klin. Med.*, 1919, LXXXVII, 338.

These two latter sediments are occasionally associated with amorphous deposits of the calcium and magnesium acid urates. These are, however, rare and need not be separately considered. In detecting the presence of the urates in a deposit, a small portion of the turbid urine is poured into a test-tube and gently heated. If urates are present the sediment will completely dissolve. These salts also give the murexid test.

(4) **Xanthin** ($C_5H_4N_4O_2$).

This substance which is chemically closely related to uric acid is rarely found as a sediment in the urine. Its chief clinical importance is found in its appearance as a urinary calculus. It crystallizes in whetstone-shaped colorless crystals which resemble those of uric acid, from which it is differentiated by its solubility on heating and in hydrochloric acid as well as ammonia. It may be chemically recognized by *Weidel's reaction*. Place a portion of the suspected crystalline deposit in an evaporating dish and dissolve by warming with a few drops of bromin water. Evaporate to dryness and place the dish containing the residue under a large beaker, allow the fumes of ammonia to fill the inverted beaker when a red or purplish-violet color will be produced in the presence of xanthin.

(5) **Calcium Oxalate** (CaC_2O_4).

This substance appears most frequently in acid urine, but may be found

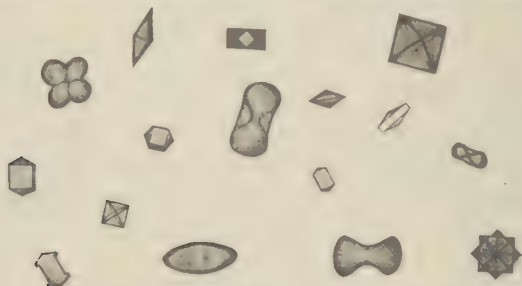


FIG. 95.—Calcium oxalate. (Hawk after Ogden.)

after the urine has undergone alkaline fermentation. If it occurs in acid urine it is associated with uric acid; if in alkaline urine, with the triple phosphates. The deposit is a colorless crystalline one having two distinct forms: (1) octahedral crystals (four-sided pyramids lying base to base); viewed from the side, these appear as squares crossed by two sharp lines, giving the so-called "envelope" crystal. (2) Dumb-bell crystals in the form of ovoid or circular disks with round margins depressed at the centers. These latter often present radial striations. Emerson has called attention to a rare type of calcium oxalate crystal which appears in the form of flat plates with parallel sides and rounded ends, looking like superimposed sheets of mica. It is characteristic of the crystallization of this substance, as of most crystalline urinary deposits, that the crystals are practically always of the same type, variations rarely appearing in the same specimen of urine.

These crystals are insoluble in acetic acid, but soluble in hydrochloric acid. This is a point of some importance, as it is occasionally difficult to

distinguish microscopically between calcium oxalate and some crystals of triple phosphate. This latter crystal is soluble in acetic acid. These crystals may be chemically identified by dissolving them in hydrochloric acid, alkalinizing with ammonium hydrate, and precipitating with ammonium oxalate.

(6) **Cystin** ($C_3H_6NSO_2$)₂.

The appearance of cystin in the urine is known as *cystinuria*. This is a condition of perverted protein metabolism which is not well understood. It may make its appearance at any period of life and in either sex, perhaps somewhat more frequently in the male. It shows a remarkably frequent hereditary character, being observed in some cases through several generations and in several children of the same parents. Many of these cases do not show any clinical characteristics, being present during their entire life without any apparent symptoms. In other cases, owing to the formation of calculi, frequent manifestations are noted and surgical intervention interposed. This condition is not very frequent, being reported only 180 times in the literature.¹

"Prior to the time when cystin was found to be a product of the disintegration of protein substances it had been conjectured, on the ground of its content in sulphur and nitrogen, that it might be a product of the intermediate protein metabolism. The explanation which von Udránzki and Baumann gave of its excretion corresponds in principle to that which is familiar to-day



FIG. 96.—Cystin. (Hawk after Ogden.)

for the appearance of conjugation products of glycuronic acid and of glyocol in urine. Like the latter, it should be normally further oxidized, and only in the presence of definite bodies should it be intercepted. These authors considered the binding substances to be the previously mentioned diamins, especially putrescin and cadaverin, which they had found in the urine and in the feces of a cystinuric patient. The formation of the diamins was supposed to be brought about by specific bacteria in the digestive tract by an extraordinary chronic intestinal mycosis. The resorbed part of the cadaverin and putrescin was supposed to protect the cystin from combustion, just as benzoic acid does glyocol, by entering into a loose combination which decomposes again after passing through the kidneys. Serious difficulties, however, have opposed themselves to this interpretation of cystinuria. First, numerous cases have been described without simultaneous *diaminuria*, and, conversely, *diaminuria* occurs in malaria, and in the conditions brought about

¹ See Fromherz, Berl. klin. Wehnschr., 1913, L, 1618; Neumann, Deutsch. med. Wehnschr., 1914, XL, 2065; Pottiez, Jour. pharm. Belg., 1920, II, 41.

by the cholera vibrios and the Finkler-Prior bacillus, without cystinuria having ever set in, any more than it does when diamins themselves are administered."

"Further investigations have shown that cystinuria is really a disturbance of the amino-acid metabolism. Of the end-products of protein hydrolysis which arise in the system, the cystinuric cannot avail himself in the normal way of the cystin, and in part excretes it; the remaining products of protein hydrolysis undergo their ordinary fate. If free monomolecular α -amino acids appear in places which are at present not well known, or if they occur there even in unusual amounts, then, unlike the normal individual, the cystinuric is unable to burn them and they leave the organism unchanged just as cystin itself does. The basic diamino acids behave in practically the same way, except that the CO_2 group is split off from them and we arrive at diaminuria" (Neuberg).

Crystals of cystin are rare in the urinary sediment. In some of the cases reported the cystin did not separate from the urine until this was acidified with acetic acid and allowed to stand for 12 hours. It crystallizes in two

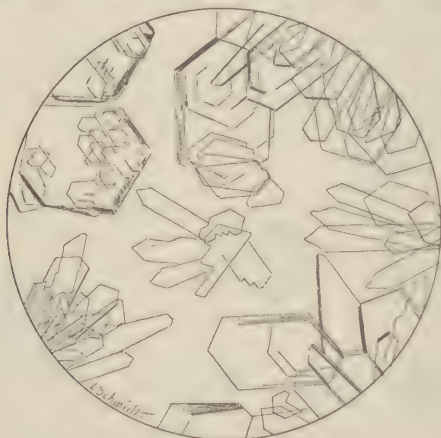


FIG. 97.—Pure leucin. (Hawk.)

forms: (1) six-sided tablets having an opalescent luster and sometimes traced with fine lines of secondary crystallization; (2) four-sided square prisms lying separately or in stellate forms. These crystals are soluble in hydrochloric acid, alkaline hydrates, and insoluble in acetic acid. These tests differentiate it from uric acid. If the urinary sediment suspected of containing cystin be treated with strong sodium hydrate solution and a few drops of benzoyl chlorid, and the mixture shaken, a voluminous precipitate of benzoyl cystin is obtained.

(7) **Leucin** ($\text{C}_6\text{H}_{13}\text{NO}_2$).

Chemically leucin is α -aminoisobutylic acid. It occurs in the urine in conditions associated with more or less marked derangement of hepatic functions (see amino acids). As found in the urine, leucin appears in the form of yellowish, highly refractile spherules, with alternating light and dark concentric layers and with radial striations. In the pure state it crystallizes

either in thin, white, hexagonal plates or as scales or rosettes of irregular shapes.

Leucin is soluble in water, acids, and alkalis, and insoluble, to a more or less extent, in alcohol. Not always do we find crystals of leucin in the sediment when the urine contains this substance. If it be suspected, the urine should be evaporated to a small bulk and alcohol added to the residue, which may then be examined for the characteristic crystals. This leucin may be identified by *Scherer's test* as follows: Some of the solid residue obtained by concentrating the urine to a small bulk is evaporated with concentrated nitric acid on a platinum crucible cover. With pure leucin the residue remains

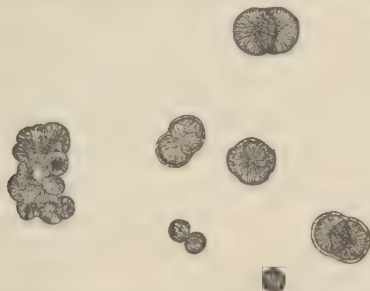


FIG. 98.—Impure leucin. (*Hawk after Ogden.*)

colorless, but as usually applied to the urine a yellowish residue obtains. This is heated with a few drops of sodium hydrate solution, when a yellowish or brown color will be observed. If further heating be applied the leucin will collect into an oily drop which rolls around on the heated surface. As leucin does not stain with Sudan-III it should not be confused with fat.

(8) **Tyrosin** ($\text{C}_9\text{H}_{11}\text{NO}_3$).

Chemically, tyrosin is p-oxyphehyl- α -amino-propionic acid. As found

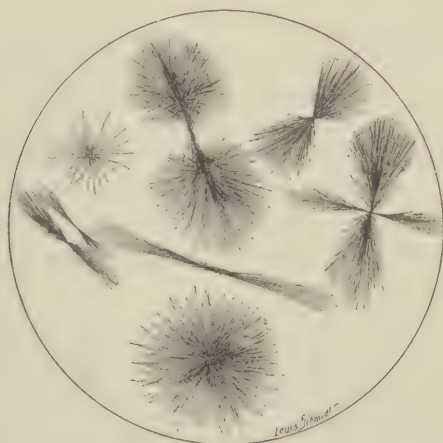


FIG. 99.—Tyrosin. (*Hawk.*)

in the urine tyrosin crystallizes in the form of fine colorless needles, which may appear black and are arranged in sheaf-like collections or rosettes. Like leucin, it may not crystallize out unless the urine be concentrated. Tyrosin

is soluble in water, acids, and alkalies, while it is slightly soluble in alcohol and insoluble in ether. As other crystals, which may appear in the urine closely resemble the tyrosin needles, it is advisable to confirm the microscopic findings by chemical tests.¹ This may be done by evaporating the urine to a small bulk, removing the fluid and dissolving the residue in water. *Mörner's test* may then be applied as follows: To this aqueous solution is added 1 c.c. of a reagent consisting of 1 c.c. of formalin, 55 c.c. of concentrated sulphuric acid, and 45 c.c. of water. If the mixture be heated to boiling a beautiful green color will be observed in the presence of tyrosin.

(9) Calcium Sulphate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$).

This is a very rare sediment, appearing only when the urine is extremely acid. The crystals appear in the form of long, thin, rhombic plates or needles which may be single, but are more frequently observed in clusters. If the sediment be boiled with hydrochloric acid and barium chlorid added, a precipitate of barium sulphate will point to the presence of calcium sulphate in the sediment.



FIG. 100.—Calcium sulphate. (Hawk after Hensel and Weil.)



FIG. 101.—Bilirubin (Hæmatoidin). (Hawk after Ogden.)

(10) Bilirubin ($\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_3$).

Bilirubin or its isomer hematoidin may appear in the urine in conditions previously discussed. The type of crystal is either a brilliant yellow or ruby-red rhomb or a yellow needle. Rarely the deposit may be in the form of a yellow granular sediment. Not infrequently small curved needle-like spines are observed projecting from the angles of the rhombic crystals. These crystals may be identified by extracting the acid urine with chloroform and applying the tests previously discussed under Biliary Pigments.

(11) Hippuric Acid ($\text{C}_9\text{H}_9\text{NO}_3$).

This substance has been observed as a sediment, although rarely. It appears in the form of semitransparent, colorless, four-sided prisms, or in long pointed rods or needles, occasionally in forms closely resembling those of the triple phosphates to be described later. These crystals are soluble in warm water, alcohol and ether and may be distinguished from uric acid by the fact that they do not give the murexid test.

¹ See Rosenbloom and Gardner, New York Med. Jour., 1914, C, 574; Gartner and Holm, Jour. Am. Chem. Soc., 1920, XLII, 1678; Folin and Looney, Jour. Biol. Chem., 1922, LI, 421.

(12) **Neutral Calcium Phosphate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$).**

This substance is found only in faintly acid or neutral urine. It is quite rare as a sediment, crystallizing in colorless needles or slender pyramids which group themselves together with their points in a common center to form rosettes or cross-shaped figures. These crystals are soluble in acetic acid and may be converted into calcium carbonate when treated with a strong solution of ammonium carbonate.

(13) **Fat.**

Under normal conditions the urine contains no free fat, but amounts varying from traces to rather large excretions may be found under pathologic conditions.¹ The excretion of fat in the urine is known as *lipuria*. This is characterized by the presence of small or large strongly refractile globules which may be stained black with osmic acid or red with Sudan-III. These globules are soluble in ether and may, therefore, be extracted from the urine by shaking out with this solvent. It not infrequently happens that the urine is contaminated with fat which may have been used in obtaining catheterized specimens or with fat coming from the bottle containing the urine. This may lead to a diagnosis of lipuria unless care be taken to exclude such a source. True lipuria has been observed in various conditions. Thus we may find after a large intake of fat in the diet, or as a therapeutic agent the so-called "*alimentary lipuria*." Pathologically, it has been observed in various cachectic conditions, in crushing injuries, especially of the bones, in eclampsia, in chronic heart disease, fatty tumors, diabetes mellitus, tuberculosis, various affections of the pancreas and liver, nephritis, and after the use of various general protoplasmic poisons. In these cases the blood may also contain an excess of fat, although this has not been observed in all cases. In fatty degeneration along the genitourinary tract, fat droplets may be seen in the epithelial cells and in the casts. Free fat is rarely found in such conditions, but occasionally it may collect in droplets which float on the surface of the urine and then constitutes true lipuria. Occasionally flat superimposed plates with notched corners (cholesterin) may be seen and may be so numerous as to justify the term "*cholesterinuria*."

In conditions associated with infection by the filaria, large amounts of fat may be present, giving rise to the appearance of an emulsion. To this condition has been given the name *chyluria*. In this form the fat may be present occasionally in large masses resembling tallow, but more frequently is seen in finer clumps of globules. The appearance of the urine is much like that of skimmed milk, but may have a reddish tinge due to the presence of blood. On allowing the urine to stand, a cream-like mass of fat will rise to the surface. It is not unusual in such cases to find the ova or the parasite in the masses of coagulated material. The excretion of the fatty material at times runs a somewhat cyclic course, being present during the day and absent at night or *vice versa*. Occasionally the excretion varies with the position of the patient, being somewhat more frequent when he is erect, and may be mark-

¹ See Sakaguchi, *Biochem. Ztschr.*, 1913, XLVIII, 1; Lawrynowicz, *Ztschr. f. klin. Med.*, 1914, LXXX, 389.

edly increased after severe exercise. This condition should be taken simply as a symptom of filariasis.

A nonparasitic type of chyluria has been observed, but its etiology is somewhat uncertain. It probably is closely related to the conditions above mentioned as causing true lipuria. It does have some relation to an increased fat diet and apparently is associated with exudation from the lymphatic vessels, as the cellular elements are largely lymphocytes.¹

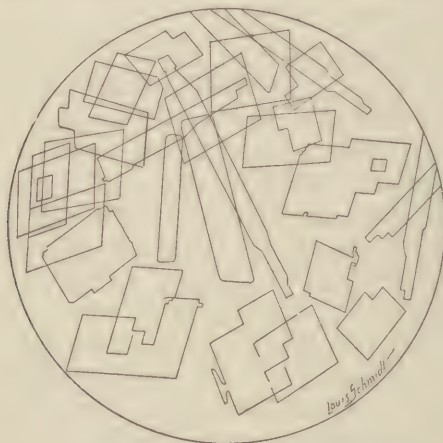


FIG. 102.—Cholesterin. (Hawk.)

(b) **Those Occurring in Alkaline Urine.**

(1) **Ammonium Urate** ($C_5H_8(NH_4)N_4O_3$).

This sediment occurs most frequently in combination with amorphous calcium phosphate and triple phosphate crystals. It is the only urate deposit found in alkaline urine, but may occur in neutral urine. It appears as a crystalline deposit of dark brown spherical masses studded with fine spiculæ, from which fact the name of "thorn-apple" crystals has been given to them. Occasionally these spheres may show concentric or radial striations. Not infrequently one observes crystals having irregular shapes, such as those of a dumb-bell or a pear.

Chemically, these crystals may be identified by dissolving in hydrochloric acid, when uric acid, which may be identified by the murexid test, will separate. If sodium hydrate be added to the dry sediment and heat applied, vapors of ammonia are given off.

(2) **Calcium Triphosphate** ($Ca_3(PO_4)_2$).

This compound is frequently found in alkaline urine, as a white amorphous flocculent deposit arranged in irregular patches. This is the usual deposit which appears in the urine when it becomes alkaline after meals. In the so-called "*phosphaturia*" the urine is always turbid when voided so that the assumption was made that an excess of phosphoric acid was being excreted. Such is found not to be the case, as a deposition of the normal phosphates of

¹ See Stern, Arch. Diagnosis, 1913, VI, 114; Young, Jour. Trop. Med. and Hyg., 1914, XVII, 241; Sanes and Kahn, Arch. Int. Med., 1916, XVII, 181; Carter, Ibid., XVIII, 541; Hymanson, Am. Jour. Dis. Child., 1916, XI, 455; Hampton, Bull. Johns Hopk. Hosp., 1920, XXXI, 20; Sano, Tohoku Jour. Exp. Med., 1920, I, 448.

PLATE X.



AMMONIUM URATE, SHOWING SPHERULES AND THORN-APPLE-SHAPED CRYSTALS.
(From *Ogden*, after *Peyer*.)

the urine must occur when the reaction becomes alkaline. It is to be said in this place that no conclusion whatever can be drawn from the separation of a substance in the sediment as regards an increase in its excretion. So many factors influence the separation or nonseparation of a sediment that a finding should not be regarded as evidence of increased formation and excretion unless quantitative chemical examination points in this direction.

Calcium phosphate is soluble in acetic acid without evolution of gas, which test may be used to show the presence of this substance in the deposits. It may be absolutely identified as a calcium compound by dissolving in acetic acid and precipitating with ammonium oxalate; the phosphoric acid radical may be proven by dissolving the sediment in nitric acid and precipitating with ammonium molybdate.

(3) **Magnesium Phosphate** ($\text{Mg}_3(\text{PO}_4)_2$).

Theoretically this compound appears along with calcium phosphate as an amorphous deposit in alkaline urine. Its amount is, however, usually less than the latter compound.

It is observed in rare cases as large, long, rhombic plates with beveled edges which closely resemble the crystals of triple phosphates. These crystals are found in cases in which not sufficient ammonia is present to form the true triple phosphate, and may be considered as transition crystals.

(4) **Magnesium-ammonium Phosphate** ($\text{Mg}(\text{NH}_4)\text{PO}_4$).

The appearance of this substance in the urine is essentially characteristic



FIG. 103.—Magnesium-ammonium phosphates. (*Hawk after Ogden.*)

of ammoniacal urine. It may very rarely be seen in amphoteric urine when ammonium salts are present in large amounts. The crystals belong to the rhombic system, appearing most frequently as triangular prisms or "coffin-lid" crystals. These may be shortened in the form of squares or one or more corners may be rounded or beveled. By refracted light a greenish tone is observed when these crystals are present. A second type of the "triple phosphate" is that of a star-shaped feathery crystal with points not unlike fern leaves.¹ These crystals are easily soluble in acetic acid and may be identified by treating with sodium hydrate and warming when ammonia is evolved.

¹ See Cavazzani, Arch. ital. biol., 1919, LXIX, 86. Schwartz (personal communication) has apparently succeeded in the experimental production of cystitis by feeding of methyl alcohol to cats.

(5) Calcium Carbonate (CaCO_3).

This substance frequently occurs in alkaline urine in association with the amorphous phosphates. It may appear as groups of amorphous material or may form large spheroidal masses with concentric radiations. Occasionally it may be observed in dumb-bell like masses which resemble somewhat the same type of calcium oxalate crystal, from which it may be differentiated by the fact that it is soluble in acetic acid with production of CO_2 , while calcium oxalate remains undissolved.



FIG. 104.—Calcium carbonate. (Hawk.)

(B) Organized Sediments.

(1) Mucoïd material.

Mucus is a constituent of practically every specimen of urine, in the form of the "nubecula." This appears in the form of small threads which branch and interlace in such a way that the entire microscopic field may be practically taken up by this material. In the meshes of the nubecular threads are observed the so-called "mucous corpuscles," which are practically identical with the ordinary leucocyte. Little significance is attached to this form of mucous threads unless a great increase is observed, when it indicates, as does mucin, a vesicle catarrh.

(2) Epithelial Cells.

Normally, the only epithelial cells found in the urine are the irregular flat cells from the bladder and urethra or the large flat epithelia seen in the urine of women and arising from the vagina. The presence of large numbers of other types of epithelial cells is always pathological and denotes an inflammatory or destructive lesion somewhere along the genitourinary tract.¹ It is a matter of more or less difficulty absolutely to identify, in all cases, the source of the epithelium found in the urine. According to Heitzmann, the positive recognition is based largely upon the size of the cell, as the shape may vary from pathologic conditions as well as from the portion from

¹ See Schkarin, Russk. Vrach, 1915, XIV, 505.

which they are derived. As stratified epithelium is found in the pelvis of the kidney, the ureters, bladder, and urethra, it is to be expected that large flat cells, cuboidal cells, or columnar cells will appear depending upon the layer from which the cell is derived. As the simple epithelium exists in the uriniferous tubules, the prostate gland, seminal vesicles, and ejaculatory ducts, the recognition of such cells will limit their origin, the size being important in determining the exact point from which they are derived. It is to be remembered, therefore, that the shape of the cell is of far less importance than its size.

The epithelial cells derived from the bladder are usually the large flat irregular cells commonly seen in all normal urine. They have a clear protoplasm and usually a small distinct central nucleus and are extremely granular. These flat epithelial cells may be single, in groups, or if the irritation is marked may occur in large sheet-like masses. The large cuboidal cells of



FIG. 105.—Epithelium from different areas of urinary tract. *a*, leucocyte (for comparison); *b*, renal cells; *c*, superficial pelvic cells; *d*, deep pelvic cells; *e*, cells from calices; *f*, cells from ureter; *g*, squamous epithelium from bladder; *h*, neck of bladder cells; *i*, epithelium from prostatic urethra; *k*, urethral cells; *l*, scaly epithelium; *m*, *m*¹, cells from seminal passages; *n*, compound granule cells; *o*, fatty renal cell. (Hawk after Ogden.)

the bladder epithelium may be seen in acute cystitis in which they are associated with large numbers of the flat cells previously mentioned. If the conditions become chronic the flat cells may entirely disappear and be replaced by cuboidal and by a few columnar epithelial cells. These latter cells are especially observed in the severe inflammatory processes in the bladder.

The large, flat, squamous epithelial cells derived from the vagina are more frequently arranged in stratified groups so that their recognition is usually simple. As these types of cells denote simple desquamation, being pathologic only when present in enormous numbers, an absolute differentiation is of little consequence clinically and, if it be so, the clinical symptoms of the case will usually clear up the decision.

The urethral epithelium very closely resembles that above described.

The cells are large and irregular, being partly flat, partly cuboidal and partly columnar. The cylindrical types of urethral epithelium may occur in the form of longer, irregular, smaller types, than those of the bladder or vagina. This type constitutes the so-called "tailed" cells, which may be derived from the pelvis of the kidney and were at one time held to be indicative of a pyelitis. Sahli regards a preponderance of such tailed cells over the flatter and more regular types as distinct evidence of trouble in the renal pelves. As these cells may be derived from other portions of the urinary tracts it is unwise to make an absolute diagnosis on such a finding. The small polygonal cells as well as tailed cells may be derived also from the ureter so that our diagnosis would necessarily rest upon findings other than such epithelium.

In the writer's opinion, it is a practical impossibility to make a positive diagnosis of a lesion in any specific portion of the genitourinary tract based entirely upon the appearance of the urinary epithelium. The points to be remembered are that we may have any type of epithelium and may have many variations in shape as well as in size. Such variations may be present in any portion of the urinary system, although distinctly renal epithelial cells are more frequently in the form of round or cubical cells somewhat larger than the leucocyte and containing a large vesicular nucleus. These latter renal cells are the only ones which seem to the writer distinctly diagnostic. They are differentiated from the similar cells arising from the ureters and prostate gland by the fact that the latter cells are about twice the size of the pus-cell, being consequently larger than the true renal cell.

Degenerative changes are frequently observed in these epithelial cells, even when examined immediately after voiding. The usual type of this degeneration is the presence of fat granules or globules, especially in the small renal cells. If the sediment be treated with Sudan-III these granules will appear distinctly red.

(3) Pus-cells.

A few leucocytes may be observed in practically every specimen of urine, especially in those from women, in which case they may be in large numbers and derived from the vagina. A marked increase, as recognized by numerous, indistinct, small, circular or irregular, granular cells, should be regarded as pathologic.¹ To this condition the name *pyuria* has been given. The simple finding of a pyuria does not necessarily indicate the point from which these cells were derived. Severe inflammatory processes anywhere along the genitourinary tract or the rupture of an abscess into the urinary tract will be associated with a pyuria, so that other features must be relied upon in deciding as to the source. As a rule, it may be said that the amount of pus is small in direct affections of the renal cortex, while disease of the urinary passages is associated with a larger number. If an abscess has ruptured into the pelvis of the kidney the number of cells may be enormous. If the pyuria be of renal origin, it will be associated with the presence of the small, round, renal epithelial cells as well as with tubular casts. Frequently leuco-

¹ See Posner, Arch. Diagnosis, 1912, V, 269; also, Pedersen, New York Med. Jour., 1913, XCVIII, 1141; MacKay, Northwest Med., 1916, XV, 402.

cytes in small numbers are found adherent to the casts or they may even be so closely grouped as to give the name pus cast to such formations. If large numbers of pus-cells appear in the course of a chronic nephritis, they indicate either an acute exacerbation of the condition or a complicating process in some other portion of the urinary tract. The sudden appearance of very large numbers of pus-cells is especially indicative of a ruptured abscess. In inflammatory processes in the pelvis of the kidney the amount of pus may vary within wide limits. In some cases the urine may be perfectly clear when voided, showing the presence of only a few pus-cells, while in others enormous numbers may appear. This paradoxical condition may be accounted for

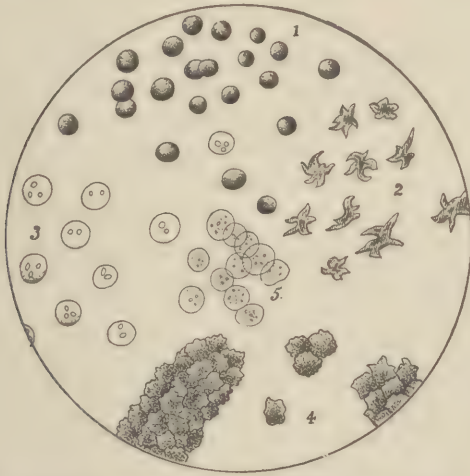


FIG. 106.—Pus corpuscles. 1, Normal; 2, showing amoeboid movements; 3, nuclei rendered distinct by acetic acid; 4, as observed in chronic pyelitis; 5, swollen by ammonium carbonate. (*Hawk after Ullmann.*)

by the possibility of obstruction of the ureter on the affected side and the later forcing out of the large numbers of pus-cells. In pyelitis the urine is usually acid, which may serve as a distinguishing point from cystitis in which the urine is almost always alkaline.

In tuberculosis of the renal parenchyma pus-cells appear very early and vary in number from a few to many thousands. This pyuria is usually constant, and is frequently associated with hematuria. The pus-cells in tuberculosis are usually of the mononuclear type instead of the ordinary polymorphonuclear form. This is not easily determined, as the degenerative processes make it somewhat difficult to distinguish the nuclear form. In such conditions the sediment should be frequently examined for the presence of tubercle bacilli and a portion inoculated into a guinea-pig. This is the only certain method of making a diagnosis of tuberculosis of the kidney. It is, perhaps, needless to add that for absolute differentiation a specimen obtained by ureteral catheterization must be examined.

In cystitis the number of pus-cells appearing in the urine will vary according to the severity of the condition, the more severe the more pus-cells.

In this condition the urine is alkaline and may be, when voided, glairy and ropy. In chronic cases of cystitis the pus-cells, although present in the bladder in large numbers, may be so degenerated by the alkalinity of the contents that practically no cells are recognizable. Here we find the appearance of a large amount of mucus, the urine being in some cases distinctly jelly-like.

In inflammatory processes of the urethra pus may be present in varying amounts. In the acute conditions the number of cells is much more numerous than in the chronic types. The recognition of the causative factor, in most cases the gonococcus, will be treated of in a later section. As the acute condition becomes subacute or chronic, the urine contains large numbers of the so-called *gonorrheal threads* which enclose numerous pus-cells.¹ These will be treated in detail later. It is sometimes a matter of clinical importance to distinguish between an anterior and a posterior urethritis. This is best done by the so-called "two-glass" test. If the first portions of the urine be collected in a receiving vessel and the later portions in a second vessel, the urine in the first vessel will be cloudy while that in the second vessel is clear in the case of anterior urethritis; while in posterior urethritis associated with the anterior type the first portion will be cloudy and the second usually so, although at times it may be clear. The reaction of the urine in both vessels will be acid unless a complicating cystitis has arisen, when the urine in the second vessel will usually be alkaline.

The appearance of the pus-cells will vary depending upon the reaction of the urine. In acid urine their structure is very well preserved, the addition of acetic acid rendering the nucleus somewhat more distinct. Their usual form is that of the polymorphonuclear neutrophile, their size varying from 7 to 12 microns. If stained² the vesicular character of the nucleus of the renal epithelial cell will absolutely differentiate it from the irregular type of pus-cell. In alkaline urine the cells swell up, lose their shape and become opaque. The addition of acetic acid usually clears them in such a way that the nucleus becomes visible, but occasionally does not. If the urine remains long in contact with the alkaline material in the bladder it becomes slimy, stringy, and gelatinous, owing to its large content in mucus. Albumin is always present so that it may be difficult to decide whether or not a true albuminuria exists.

As the pus-cells may undergo such marked change when in alkaline urine and be converted entirely into a gelatinous mass in which corpuscles cannot be detected, certain tests must be applied for positive recognition of pus in such cases.

Vitali's Test.

Acidify the urine with acetic acid and filter. Treat the material on the filter with a few drops of tincture of guaiac, when a deep blue color will appear in the presence of pus. If the material is not filterable, as happens when the purulent material is extremely gelatinous, place a portion of this slimy urine in a test-tube and allow a few drops of tincture of guaiac to flow

¹ See Broadman, New York Med. Jour., 1913, XCVIII, 28; also, Thomas, Am. Jour. Med. Sc., 1913, CXLVI, 696.

² See Fittipaldi, Gaz. d. Osp., 1915, XXXVI, 1265.

upon the surface. If pus be present a distinct blue line of contact will be observed.

Donné's Test.

A portion of the urinary sediment in a centrifuge tube is treated with a few drops of concentrated solution of sodium hydrate. If pus be present an extremely viscid gelatinous mass will be obtained. If this mixture be heated, it will dissolve, according to Müller, with the formation of β -nucleinic acid.

If the pus-cells be treated under the microscope with a few drops of Lugol's solution, they will take a mahogany-brown color owing to the presence of glycogen.

Enumeration of Pus-cells.

Such a procedure is not a part of the ordinary routine examination of urine. It is, however, sometimes advisable, as it permits of a decision regarding the presence or absence of a true albuminuria. If the latter exists, casts and renal epithelial cells will usually be present so that a diagnosis is often possible without a count of the cells.

Technic.

A portion of the 24-hour specimen of urine is thoroughly shaken to bring the corpuscles into suspension. This turbid fluid is then drawn up to the upper (11) mark of the leucocytometer, a drop placed upon the glass slide and the cells counted as described under Blood. If more than 30,000 per cmm. are present, it is advisable to dilute five times with 3 per cent. sodium chlorid solution. For each 100,000 leucocytes per cmm. of urine 0.1 per cent. of albumin is assumed to be present, according to Wunderlich.

(4) Red Blood-cells.

The presence of red blood-cells in the urine is known as *hematuria*. This condition should be sharply differentiated from hemoglobinuria as the clinical significance is entirely distinct. Blood may be found in the urine in a variety of conditions. Thus in the more malignant types of the acute infectious fevers hematuria is frequently observed. Likewise, in scurvy, hemophilia, purpura, leukemia, and Werlhof's disease, the kidney may be so markedly affected that hematuria obtains.

In the hematuria of purely renal origin we find both acute and chronic congestions as well as inflammatory processes in the kidney associated with this condition.¹ In the more acute types of nephritis, hematuria is so common that the name "hemorrhagic nephritis" is frequently applied. Such cases are especially observed after poisoning with cantharides and phenol derivatives.² The chronic parenchymatous type of nephritis is, according to Weigert, always hemorrhagic in type, the number of red corpuscles being an indication of the intensity of the process. In malignant growths of the kidney, tuberculosis, renal calculus, and cystic degeneration of the kidney, hematuria is especially common; while in infection with certain parasites, such as the filaria, echinococcus, and the distoma hematobium, hematuria is relatively frequent although few cases of these conditions are seen.

¹ See Randall, Jour. Am. Med. Assn., 1913, LX, 10.

² See Belkowsky, Rev. de. méd., 1913, XXXIII 663, for a discussion of hematuria caused by urotropin.

Hematuria may also be observed as a result of lesions or disease of any portion of the urinary tract. Thus stone in the ureter or urethra, tumors, ulcers, and parasites of the bladder, urethritis, prostatitis or injury during catheterization may also be associated with the appearance of red cells in the urine.

A further type of cases in which hematuria occurs is known as the *functional or idiopathic hematuria*. In this class of cases no definite lesion has been found to account for the condition. It has been called "Gull's renal epistaxis," "essential renal hematuria," "angioneurotic hematuria," "renal hemophilia" and "renal aneurysm." The lesion, whatever it may be, is usually unilateral and the attacks appear at variable intervals. Some of the cases recover without any treatment after one or two profuse hemorrhages, while others require extensive treatment of the nervous system.¹

In the diagnosis of a hematuria it is important to observe the appearance of the urine both with the naked eye and with the microscope. The urine is turbid, and varies from a light, hazy, "smoky" appearance to a bright-red or deep-brown color. The red cells appear in various stages of preservation. In some cases the normal yellow color of the cell will be quite distinct while in others the color will be entirely washed out. If the urine be particularly concentrated many crenated forms will also be observed.

The blood-cells may exist singly and scattered, or may be grouped in large masses forming distinct clots or adherent to tube casts forming the so-called blood casts. In true renal hematuria the blood is intimately mixed with the urine, the individual corpuscles usually appearing as pale shadows or "ghosts." In hemorrhage from the bladder the urine may show the presence of blood-clots of irregular form and size. If the two-glass test be applied, the second glass will contain the more blood, while in hematuria of renal origin both glasses will show equal amounts. In some cases clots of blood in distinct casts are seen. In the chronic parenchymatous nephritis clots are rarely present, while in malignant disease of the kidney clots are relatively common.

It is important in making a diagnosis from the presence of blood that extraneous sources of blood-cells be excluded. If the blood be of renal origin it will be associated with the presence of casts and epithelial cells while no such elements will be present from a hemorrhage lower down in the genito-urinary tract. Albumin will also be present in more or less amount. It has been stated that if the blood be derived from other than renal sources, the clear supernatant fluid in the centrifuge tube will be albumin-free. The writer has convinced himself that this is an error, as he practically always obtains faint albumin reactions in cases of hemorrhage other than renal.

¹ See Braasch, Jour. Am. Med. Assn., 1913, LXI, 936; Kemble, Wash. Med. Ann., 1914, XIII, 150; Kretschmer, Interstate Med. Jour., 1914, XXI, 1256; Wulff, Hospitalstid. 1914, LVII, 1465; Kornmann, Münch. med. Wchnschr., 1915, LXII, 1422; Krotoszyner, Calif. State Jour. Med., 1915, XLII, 415; Lipschitz, Cor. Bl. f. Schweiz. Aerzte, 1915, XLV, 1473; Wildbolz, Ibid., 1586; Sanders, N. Y. Med. Jour., 1916, CIII, 1226; Gast, Deutsche med. Wchnschr., 1916, XLII, 1166; Kretschmer, Jour. A. M. A., 1917, LXVIII, 508; Wiseman, Am. Jour. Med. Sc., 1917, CLIV, 264; Escudé, Jour. d'Urol., 1919, XIII, 381; Bennett and Frankau, Quart. Jour. Med., 1920, XIII, 195; Chute, Boston Med. & Surg. Jour., 1920, CLXXXII, 623; Sfakianakis, Deutsch. med. Wchnschr., 1920, XLVI, 690; Vinson, Florida Med. Assoc. Jour., 1920, VII, 55; Andalo, Policlinico, 1921, XXVIII, 110; Macalpine, Brit. Med. Jour., 1921, I, 631; Fullerton, Ibid., 923; Bruni, Rif. Med., 1921, XXXVII, 697; Keppeler, Beitr. z. klin. Chir., 1921, CXXIII, 236; D'Agata, Policlinico, Surg. Sec., 1921, XXVIII, 325; Pizzetti, Ibid., 347; Bufalini, Arch. Ital. di Chir., 1921, IV, 540.

(5) Casts.

True casts are moulds of the uriniferous tubules. Their mode of formation is not entirely clear. Undoubtedly a colloid substance is thrown into the lumen of the tubule and later solidifies forming a distinct cast of that particular tubule. In this process of hardening the material may enclose cells of different types which are, also, present in the tubule. Whether this coagulable material is derived from the blood as a transudate, whether it be a secretion of the epithelial cells which have become pathologic, or whether it be material arising from degeneration of renal cells is not at present settled, the latter source being the more probable.¹ In the urine we find true casts of the renal tubules, as well as pseudo casts which have nothing in common with the true type of these pathologic formations.

True Casts.

Hyaline Casts.

The true hyaline casts are pale, transparent, homogeneous cylinders with rounded ends. Their size may vary from a very small fragment to one several mm. in length. In diameter they may be narrow or broad. As a rule, little



FIG. 107.—Hyaline casts. One cast is impregnated with four renal cells. (*Hawk.*)

difference is clinically made between these various types of hyaline casts, but the broader types seem to the writer to be somewhat more significant than do the narrower ones. The characteristics of the true cast are their cylindrical appearance, their sides being parallel and usually straight, although they may at times be observed in typical tortuous forms. They are never tapering at the ends, but may show an irregular outline at one or both ends, but the length and the parallel sides will usually differentiate them.

¹ See Erdman, *Jour. Am. Med. Assn.*, 1912, LIX, 1952; Posner, *Ztschr. f. Urol.*, 1921, XV, 113.

The pure hyaline casts are perfectly homogeneous and free from granules. Such types are, however, not frequently observed as very fine granules may almost always be detected embedded in the surrounding homogeneous material. There may be even inclusions of epithelial, renal, blood-, or pus-cells, so that the gradations between the pure type of hyaline casts and many of the other varieties are outlined with difficulty. It should be stated at this point that a distinction exists between hyaline casts with enclosures of cells to such an extent that the cast is named from the variety of cell present and the type of pseudo cast in which groups of such cells are massed so as to form an apparent cast, but which do not have any definite matrix. The true hyaline cast is soluble in acetic acid and may be stained yellow with Lugol's solution.

These hyaline casts are not always easy to find in the sediment. In examining the urine for the presence of casts the light should be shut off as much as possible and a low-power lens used. With the use of the high power the field is limited and one is not so apt to observe the cast as with the low power. It is, however, always advisable to examine a cast, first seen with the low power, under the high power, so that the decision may be much more definite as to whether the cellular elements are real inclusions or simply material resting upon the true cast. The same is to be said regarding the presence of granules in the hyaline cast.

Significance.

Regarding the significance of hyaline casts in the urine, it is to be said that they occur in any condition in which the kidney is altered by circulatory, toxic, or inflammatory disturbances. They are not pathognomonic of any one condition and may be found as a result of simple functional disturbance. A few hyaline casts may be found in practically every urine, providing sufficient search is made. Any undue strain, such as running for a car in one who is not used to such exertion, may be sufficient to add quite a number of hyaline casts to the urine. In many thousand urine examinations made in the writer's laboratory, it has been rather the unusual thing not to find an occasional hyaline cast. It would seem, therefore, that no significance whatever should be attached to the presence of an occasional hyaline cast. When, however, these casts become very numerous they should then be interpreted as meaning a disturbance of the kidney, although the absence of other types would rather speak against a marked pathologic change. In diabetes mellitus, "showers" of casts are especially observed preceding the appearance of coma (Külz).

Granular Casts.

These are modifications of the true hyaline cast in the sense that fine or coarse granules are found in the matrix of the hyaline cast. Several types of granular casts are observed. The granules may be very fine, very coarse, or may be distinctly composed of denegenerated epithelial cells. The fine as well as the coarse granules are undoubtedly derived from the renal epithelium, which has degenerated completely. The coarser the granules the more severe the inflammatory process.

These granular casts vary in shape and in size, but are usually shorter than the hyaline type. To these granular casts may be attached various cells so that it is difficult to tell whether the cast is really a true granular or a cellular one. In some cases these cellular inclusions may undergo fatty degeneration giving a much higher refractility to the specimen. Not infrequently one observes hyaline casts which are distinctly granular in one portion while the other is perfectly homogeneous.

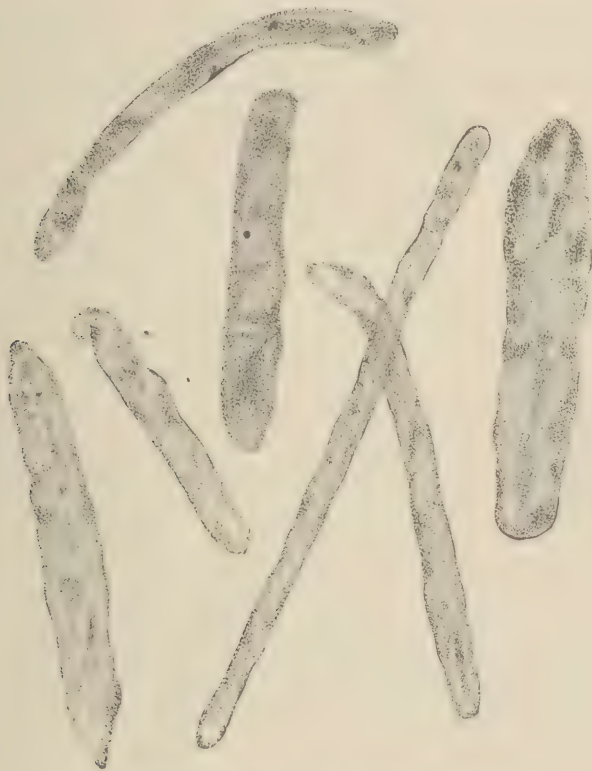


FIG. 108.—Granular casts, $\times 225$. (Tyson.)

The so-called *brown granular casts* appear to be almost entirely degenerated epithelial cells, although the coloring matter is probably hemoglobin. The fact that the hyaline matrix cannot be distinctly made out does not argue against this type being truly hyaline in character, although the matrix is completely saturated with the pigment.

Waxy Casts.

This type is very refractile, transparent, and either perfectly colorless or showing a slight shade of yellow. Usually they are very long and broad and may be either straight or curved. The ends show a very distinct fracture while the cast itself may show a tendency to split transversely. Their appearance is, therefore, that of ordinary wax. They may have any type of

cellular element attached and may show marked fatty degeneration. Some of these casts show the amyloid reaction while many of them do not.

Waxy casts were at one time believed to be pathognomonic of amyloid degeneration of the kidney. It is true that they do appear earlier in this type of kidney lesion, but it is to be remembered that they occur in all varieties of chronic kidney disease. They are usually of bad prognostic omen as they indicate a very advanced process.

Fibrinous Casts.

These are very highly refractile, transparent, and always of a yellowish or brown color. They may be granular and have various cellular inclusions. Their shapes vary as do those of the hyaline types and they show a tendency to become fractured, the fracture usually being ragged, while in the waxy cast it is sharp-cut.

Fibrinous casts usually appear in the acute renal conditions and disappear when this condition clears up. They do not have, therefore, the grave significance of the waxy cast and should be sharply differentiated. These casts are not composed of fibrin as their name would indicate, but are so called on account of their brownish color, which resembles fibrin.

Epithelial Casts.

These casts are true hyaline casts which include so many renal epithelial cells that the hyaline matrix may be lost. For the name epithelial cast to be accurate it is not necessary that more than a few cells be present. The cells may be well preserved or show marked fatty or granular degeneration. The nuclei

of these cells are round and vesicular so that they may be easily recognized.

Distinct gradations exist between the true epithelial cast and the coarsely granular and fatty cast. This type of cast is indicative of a severe destructive lesion of the kidney epithelium.

Fatty Casts.

These casts are masses of epithelial cells which have so markedly degenerated that little is recognized beyond the original outline of the cell and the numerous fatty globules contained therein. They may be yellowish or black in color, the globules being soluble in ether and staining black with osmic acid or red with Sudan III.

Blood Casts.

These are casts including large numbers of blood-cells. The casts are formed within the tubules of the kidney, the cells occasionally being very pale. These casts indicate a serious advanced lesion of the renal parenchyma.

Pus Casts.

These like the other types of casts are true hyaline casts with enclosures of pus-cells. They are formed within the tubules of the kidney and usually



FIG. 100.—Epithelial casts. (Hawk.)

PLATE XL



WAXY CASTS TREATED WITH IODINE. (*Tyson.*)

indicate an acute pyelonephritis. For the differentiation of these casts from epithelial casts it is advisable to add acetic acid to the sediment when the typical polymorphous character of the nucleus will distinguish the pus-cell from the epithelial cell with its vesicular nucleus. Moreover, the pus-cell is much more spherical than is the epithelial cell.

Cylindroids.

It is not infrequent to find in the urine formations which resemble the true hyaline casts to a marked degree. They, however, differ in the fact that at one or both ends they taper off into a point which may be prolonged into a distinct thread. If, however, these ends are broken off, as may occur in the centrifugation, it is a practical impossibility to distinguish them from a hyaline cast. They are both found in the urine under the same conditions and their significance is practically the same. From the chemical standpoint

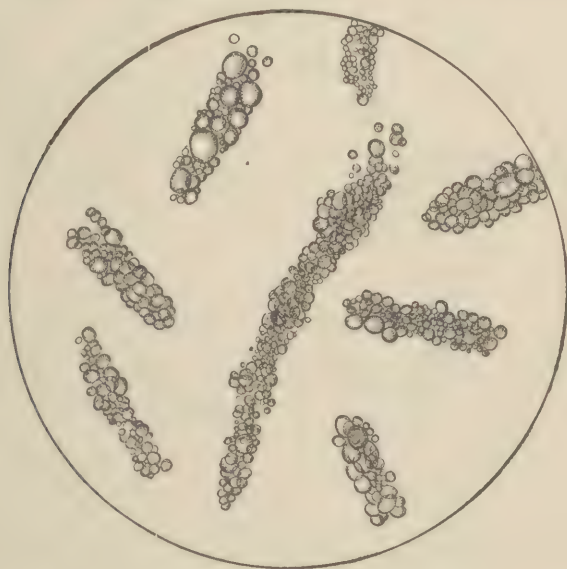


FIG. 110.—Fatty casts. (*Hawk after Peyer.*)

they appear similar to the hyaline casts, their origin, therefore, being presumably in the renal parenchyma. If these bodies are true mucin and are insoluble in acetic acid, their origin is more probably in the bladder.

A second type of cylindroid appears in the urine in the form of long tapering transparent shreds. They very much resemble ribbon which varies in diameter and may show under high power a distinctly fibrillar structure. These threads largely compose the nubecula. They are much longer than the hyaline cast and considerably narrower so that confusion should not arise. In cases of gonorrhea one finds mucous shreds which may vary from a few mm. to 1 cm. in length and yellowish or pure white in color. In the meshes of these shreds one finds embedded large numbers of pus and epithelial cells. These should be sharply differentiated from the true cast by their larger size

and typical mucoid character. Frequently they may be observed by the naked eye in large numbers.

Pseudocasts.

Not infrequently do we find in urine crystalline material arranged in masses much resembling casts. The most important of these are uric acid and the urates. It is true that any cast in a concentrated urine may become covered with urates so that the true nature of the cast becomes indefinite. If the slide be warmed these pseudourate casts will disappear, while the true casts will remain. Masses of bacteria, pus-cells, epithelial cells and blood-cells may so group themselves as closely to resemble true casts. As a rule such masses will show irregular outlines and no evidence of a distinct matrix. Moreover, the use of an old slide upon which there may be many scratches



FIG. III.—Blood, Pus, Hyaline and Epithelial Casts. (Greene.)

a, Blood casts; b, pus cast; c, hyaline cast impregnated with renal cells; d, epithelial casts.

should be avoided as the writer has seen several instances in which supposed casts were found to be due to such scratches.

Cylindruria.

This is the name given to the appearance of casts in the urine. As a rule, it should be said that the presence of a few hyaline casts is not of particular moment unless associated with other evidences of marked renal disturbances. While albuminuria and cylindruria usually go hand in hand, yet we do find cases in which one exists without the other.

It is undoubtedly true that casts indicate a disturbance of the renal epithelium. This, however, need not be anything more than disturbed nutritional or circulatory conditions. However, when the true hyaline casts are present in large numbers and when many other types of casts also exist, then a dis-

PLATE XII.



Katharine Mill.

MUCOUS THREADS IN URINE. (UNSTAINED SPECIMEN)

tinct pathologic lesion of the kidney must be assumed.¹ As a rule, the granular types of cast are observed in the chronic processes, while the cellular forms are more usually present in the acute conditions. This rule, however, is not invariable, so that it may generally be stated that no type of cast is pathognomonic of any single condition. In this connection we should remember that recent work, especially that of Cabot, has shown that it is unwise to base a diagnosis of a kidney lesion upon the finding even of both albuminuria and cylindruria. So much discrepancy was shown to exist between the urinary and autopsy findings that one must remain in doubt as to whether it is possible to make a definite diagnosis unless clinical symptoms other than urinary are made the basis of a diagnosis. On the other hand,



FIG. 112.—Cylindroids. (*Hawk after Peyer.*)

some of the most typical cases of nephritis, as shown postmortem, gave absolutely no indication in the urine that such condition existed. We are, therefore, face to face with the proposition that urinary examination must be in any case simply one of the diagnostic links. This fact is of special importance in life insurance examinations, as most companies absolutely refuse insurance to one who has ever shown albumin or casts in the urine. This would seem to the writer not only very short sighted, but based upon an absolutely erroneous idea of the importance of albumin and casts in the urine of one who showed absolutely no clinical signs of renal involvement. Remembering that albumin and casts may not appear, even though the kidney be seriously affected, it would seem just as plausible to refuse life insurance because these substances were not present. In this connection the writer would say that only when the urine is considered as a whole may definite conclusions be made regarding any type of renal disease. The clinician, who

¹ See Barringer and Warren, *Arch. Int. Med.*, 1912, IX, 657; also, Posner, *Berl. klin. Wchnschr.*, 1913, L, 2040; Minerbi, *Policlinico*, 1914, XXI, 1667; Eigenberger, *Zentralbl. f. inn. Med.*, 1920, XLI, 354.

is thoroughly familiar with the course of the case, is the only one capable of interpreting the findings of the laboratory, so that it should be an unvarying rule for a laboratory worker to avoid diagnostic remarks unless he is thoroughly *en rapport* with the patient. The writer does not wish to be interpreted as stating that a diagnosis of renal disease may never be made from an examination of the urine, but he wishes to impress upon his readers that both albuminuria and cylindruria may occur without direct kidney disease or may not appear when such is present.¹

(6) Spermatozoa.

Spermatozoa are frequently observed in the urine of healthy adults, especially after intercourse or nocturnal emissions. In females they may also be observed as an evidence of intercourse, which fact is of some importance in cases of suspected rape.

Pathologically, they may be found in cases of marked constipation, when the pressure of the impacted feces upon the seminal vesicles may induce an emission. In occasional cases of cystitis, associated with stricture, these bodies may be observed as reported by Simon. In cases of epilepsy and hysteroepilepsy, as well as in spinal disease following vertebral fractures and dislocations, spermatozoa are not infrequent. Masturbation and venereal excess frequently lead to almost constant *spermatorrhea*. Their occurrence in cases of prostatitis will be discussed in the section on Semen.

(7) Tissue Fragments.

It is not infrequent to find shreds of tissue in the urine, which may throw some light upon a pathologic condition. In cases of carcinoma of the bladder, more rarely of the kidney, true malignant tissue may be obtained, which may permit of a tentative diagnosis, although the material is usually too necrotic to make an absolute diagnosis possible.

(8) Bacteria.

It should be stated in the beginning of this discussion that an examination of the urine for bacteria should be made only upon specimens obtained with the greatest possible precaution and preserved in absolutely sterile vessels.² Soon after the urine is voided, especially if it remains in contact with the air, large numbers of saprophytic organisms may be found which, of course, did not exist in the original urine. In obtaining a specimen from the male it is not always necessary to catheterize the patient. If the surface of the glans and the orifice of the meatus be carefully washed with bichlorid solution followed by sterile water and the first portion of the urine voided be thrown away, the last portion may be collected in a sterile vessel and later put in work. With female patients, however, it is absolutely essential that catheterization be performed. The external genitalia and especially the orifice of the urethra are well washed with green soap and water. The opening of the urethra is then dried with sterilized cotton pads which are soaked in boracic acid. A sterilized glass catheter, whose external end is covered with a rubber tube about four inches long and large enough to fit loosely over the catheter is

¹ See MacLean, Med. Res. Comm., Special Rep. Series 43, 1919; Dublin, Am. Jour. Hyg., 1921, I, 301.

² See Hort, Jour. Hyg., 1914, XIV, 509.

then inserted, care being taken that it touches only the orifice of the urethra. The urine is allowed to flow freely for a short time when the last portion is collected in a sterile vessel, the rubber tube being previously removed (Kelly). Cultures are then made from the urine and the remainder centrifuged in a sterile closed tube in order to throw down any bacteria which may be present. It is sometimes advisable, in order to diminish the specific gravity of the specimen, to add an equal volume of 95 per cent. alcohol and centrifuge the mixture. Practically all of the bacteria present will then be found in the sediment.

The supernatant fluid is then removed by quickly inverting the tube and allowing the fluid to run out. The sediment, by this manipulation, will usually remain in the smaller portion of the tube. Smears are then made upon a glass slide and dried first in the air and then over the flame. It is not always the simplest matter to prepare smears which will remain after treatment with the staining solution, as the urea and salts of the sediment may be removed by the washing and carry with them the bacteria. If pus-cells are present, satisfactory smears are usually obtained; but if such conditions do not exist it is advisable to add a solution of egg albumin to the sediment before drying over the flame.

The methods of staining the sediment for the various bacteria will depend entirely upon the organism supposed to be present. As a rule, a preliminary examination is very satisfactorily made by treatment with Löffler's methylene-blue solution, which stains practically all organisms. If the tubercle bacillus is suspected it may be detected in exactly the same way as outlined under Sputum by staining with carbol-fuchsin solution. Should one suspect the presence of the gonococcus¹ this may be stained, as described in the next section, by Gram's method. Outside of these two types of organisms, it is almost impossible to differentiate the bacteria of the urine by staining methods.

If the urine be collected with the precautions mentioned above, any organisms found must be attributed to their presence in the urine as voided. In this connection we must remember that the presence of the tubercle bacillus *does not necessarily indicate* tuberculosis along the genitourinary tract.² Tubercle bacilli are found in the urine in cases of miliary tuberculosis and have been reported in pulmonary tuberculosis, although it is more frequent to find them as evidences of local tubercular conditions. Of course, if large numbers of pus- and blood-cells be present along with tubercle bacilli, the diagnosis is usually certain. A word of caution is, however, necessary at this point. The smegma bacillus grows in abundance on the external genital organs and its morphological and staining characteristics may closely resemble those of the tubercle bacillus.³ If the proper precautions be observed,

¹ This must not be confused with the micrococcus catarrhalis, which not infrequently is the etiologic factor in genitourinary inflammations.

² See Kielheuthues, *Folio urol.*, 1912, VII, 191; Beer, *Am. Jour. Med. Sc.*, 1917, CLIV, 251; Humbert, *Rev. Méd. de la Suisse Rom.*, 1917, XXXVII, 35; Watson, *Am. Jour. Med. Sc.*, 1918, CLVI, 636; Spooner, *Jour. Med. Res.*, 1918, XXXIX, 59; Fragale, *Policlinico*, 1922, XXIX, 511.

³ See Gautier, *Jour. d'Urol.*, 1914, V, 161; Brereton and Smith, *Am. Jour. Med. Sc.*, 1914, CXLVIII, 267; Churchman, *Ibid.*, 722; Editorial, *Jour. Am. Med. Assn.*, 1915, LXIV, 348; Brown, *Ibid.*, 886.

as they should be, no differentiation is necessary, bacilli showing the true morphological and staining character of the tubercle bacillus can be only this organism, as extraneous bacteria have been avoided. In the clinical laboratory, however, one may never be sure whether the proper precautions have been taken, so that absolute methods of differentiation (see Sputum) should be part of the technic unless the worker absolutely knows that contamination was avoided. A second point regarding the tubercle bacillus is that it may not be found by microscopic examination even after repeated attempts. Under such conditions the wisest course to pursue is the inoculation of a guinea-pig with the washed urinary sediment. The obtaining of the urine must in this case be absolutely accurately done by observing every precaution to prevent contamination. The reason for this is not because contaminating organisms will cause lesions similar to those of the tubercle bacillus, but because such secondary invaders may so infect the animal that death results from causes other than those for which we are looking. The washed sediment is injected intraperitoneally and the animal kept under observation for three weeks to one month unless death results previously.¹ At the end of this time the animal is killed and a postmortem examination made for evidences of tuberculosis. The retroperitoneal glands, spleen, and liver are the especial organs to show such lesions. These organs should be sectioned, portions run through the regular pathologic routine, and sections examined microscopically. A finding of tuberculosis in this way is unequivocal and is the quickest way in the long run of making a positive diagnosis, although a single examination may show the presence of tubercle bacilli in the urine, but rarely such is the case.²

Having found the tubercle bacilli in the urine, we are confronted with the question of the part affected. As a primary tuberculosis of the bladder is rare we may usually assume the seat of the difficulty to be the kidney, although if evidences of cystitis be present a combination may exist. As the symptoms of genitourinary tuberculosis are frequently vesical in origin, a kidney lesion may not be suspected, but should be assumed until the contrary is proven. Thanks to the introduction of methods of cystoscopic examination and especially ureteral catheterization, we are in a better position to make a positive diagnosis of renal tuberculosis and exclude that of bladder origin. An interesting point regarding tuberculous cystitis is that the urine, although frequently containing large amounts of pus, is practically always acid in reaction. Moreover, this pus is frequently sterile in tubercular cystitis. It is not the province of the writer, at the present time, to outline methods of differential diagnosis of various conditions; he will refer, therefore, to works on genitourinary diseases for the various types of cystitis and their clinical differentiation. Suffice it to say at this point that cystitis may be an ascending or a descending one and should always be correlated with the associated

¹ Morton (*Jour. Exper. Med.*, 1916, XXIV, 419) has shown that the use of guinea pigs, which have been exposed to X-rays, permits a diagnosis in from 8 to 10 days.

² See Bryan, *New York Med. Jour.*, 1913, XCVIII, 20; also, Keene and Laird, *Am. Jour. Med. Sc.*, 1913, CXLVI, 352.

PLATE XIII.

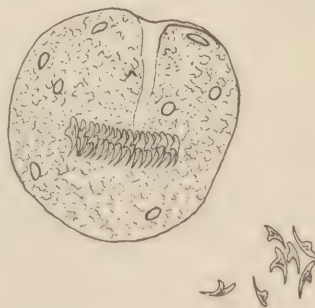


CYSTITIS DUE TO COLON BACILLUS. (METHYLENE BLUE STAIN.)

condition.¹ It is usually a simple matter to determine the presence of the gonococcus in the urethral discharge, but it is far from easy to demonstrate this organism in the case of gonorrheal cystitis. Under such conditions the symptoms and the association with an existing gonorrhea would furnish the decisive clue.

Bacilluria.

By this condition is meant the presence in the freshly voided urine of so many organisms that the urine is distinctly cloudy. These organisms are usually those associated with a mild cystitis or may be those of an existing general infection.² In cases of persistent bacilluria, we may find a true renal origin, which is largely associated with the presence of the typhoid and colon bacillus. As has been well established, the typhoid organism may be excreted for months after the patient is convalescent so that it becomes necessary to use strict measures to disinfect all urine of typhoid patients. The colon bacillus is at present assuming so much importance in clinical work that cases are being almost daily recognized which may be directly traceable to the colon bacillus and not to the typhoid as usually assumed. It is necessary, therefore, for the laboratory worker especially, and, where possible, for the general practitioner to be able to recognize each of these organisms when present either in the feces, urine, milk, or water-supply (see Feces). A second general class of cases associated with bacilluria are those of urethritis and prostatitis. Usually there is a secondary cystitis arising from the same organism or, at least, the resistance of the bladder has been so far reduced that these organisms which find their way into the bladder develop profusely therein.



Katharine Hill

FIG. 113.—Scolex and hooklets of *taenia echinococcus* in urine.

¹ See Peterkin, *Urol. and Cut. Review* (Tech. Supp.), 1915, III, 188; Beeler and Helmholtz, *Am. Jour. Dis. Child.*, 1916, XII, 345; Mathers, *Jour. Infect. Dis.*, 1916, XIX, 416; Curtis, *Jour. A. M. A.*, 1916, LXVI, 1456; Dick and Dick, *Arch. Int. Med.*, 1917, XIX, 493; Thomas, *Am. Jour. Med. Sc.*, 1917, CLIII, 701; Schwartz, *Am. Jour. Dis. Child.*, 1917, XIII, 420; Eisendrath and Schultz, *Jour. Med. Res.*, 1917, XXV, 295; *Jour. A. M. A.*, 1917, LXVIII, 540; Crabtree and Cabot, *Ibid.*, 589; Quinby, *Ibid.*, 501; Coyon and Lemièrre, *Bull. Soc. Med. Hop. Paris*, 1919, XLIII, 743; Barney and Welles, *Jour. Am. Med. Assoc.*, 1920, LXXIV, 1499; van Rijssel, *Nederl. Tijdschr. v. Geneesk.*, 1920, II, 590; Pages, *Semana Med.*, 1921, XXVIII, 296; Hyman and Mann, *Jour. Am. Med. Assoc.*, 1921, LXXVII, 1012.

² Freifeld (*Berl. klin. Wchnschr.*, 1913, L, 1761) reports the finding of diphtheria bacilli in urine. Rosenow (*Jour. Infect. Dis.*, 1909, VI, 296) and Townsend (*Jour. Am. Med. Assn.*, 1913, LXI, 1605) report the pseudo-diphtheria bacillus as a causative factor of cystitis. See, also, Curtis, *Jour. Am. Med. Assn.*, 1915, LXIV, 270; Surg., Gyn. and Obs., 1915, XXI, 423. Barber and Draper, *Jour. Am. Med. Assn.*, 1915, LXIV, 205; McGowan, *Ibid.*, 226; Cunningham, *Ibid.*, 231; Koll, *Ibid.*, 297; Greeley, *New York Med. Jour.*, 1915, CI, 250; Hoover, *Interstate Med. Jour.*, 1915, XXII, 163; Kretschmer, *Ibid.*, 173; Dick, Dick and Rappaport, *Jour. Inf. Dis.*, 1916, XVIII, 216; Morishima and Teague, *Ibid.*, 1917, XXI, 145; Herrold and Culver, *Ibid.*, 1919, XXIV, 114; Schmidt, *Ill. Med. Jour.*, 1919, XXXVI, 188 and 241; Wagner and Davison, *Bull. Johns Hopk. Hosp.*, 1921, XXXII, 50; Cabot, *New York State Jour. of Med.*, 1921, XXI, 35; Cornwall and Lafrenais, *Indian Med. Gaz.*, 1921, LVI, 325; Thomson, *Glasgow Med. Jour.*, 1922, XCVII 82; Cecil and Hill (*Jour. A. M. A.*, 1922, LXXVIII, 575) report the isolation of actinomyces from urine.

(9) Parasites.

Various types of parasites are observed in the urine. Thus the trichomonas vaginalis has been found by K nstler, Miura, and Dock. Ameb  have been found by B lz, J rgens, Wijchoff, and by Musgrave and Clegg. Various portions of hydatid cysts are frequently observed, among which we find the echinococcus hooklets and fragments of membrane. Nematode worms, especially the filaria sanguinis hominis, are present in cases of chyluria, while the anguillula aceti or "vinegar-eel" has been reported, especially by Stiles, while Billings and Miller report its presence as a possible contamination from the bottle in which the urine was collected.¹

Eggs of the schistosomum h matobium are not infrequently observed together with large numbers of red cells in cases of bilharziasis. This worm as well as its ova will be discussed in the section on Blood. Stuertz has reported the findings of the egg of eustrongylus gigas in the urine in a case of chyluria.

V. CALCULI

Concretions of a more or less hard and dense character are prone to form in the urinary passages. These bodies are termed, according to their size and location, sand, gravel, stone, and calculi. These formations consist of accretions of any of the various crystalline or amorphous sediments previously mentioned, the type of stone depending upon the reaction of the urine.

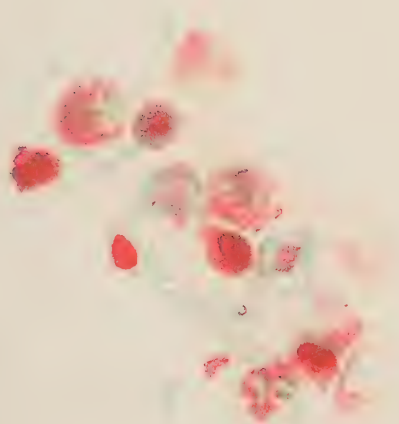


FIG. 114.—Ova and miracidium of schistosomum hematobium, $\times 300$: A, Ovum as seen in urine; B, the same after addition of water; C, miracidium. (Tyson after Railliet.)

These calculi are formed by the deposition of the crystalline material around a definite nucleus, which usually consists of organic material, such as fibrin, blood, desquamated epithelial cells, mucus, or even a crystal of uric

¹ King (Jour. Am. Med. Assn., 1914, LXIII, 2285) reports a case of Myiasis of the urinary passage due to the presence of the larva of the small "latrine fly" (*fannia scalaris*). See, also, Leon, Jour. Parasitol., 1921, VII, 184.

PLATE XIV.



STAPHYLOCOCCUS CYSTITIS. (LEISHMAN STAIN.)

acid or calcium oxalate.¹ It is very difficult to decide as to the reason for the deposition of this material in the form of a renal stone. The growth of the calculus takes place by accretion, the deposition of successive layers of material occurring around the original nucleus. The material of which the stone consists will usually be of one kind, so that we speak of uric acid or phosphate calculi, for instance, while, occasionally, mixed calculi may be formed by the deposition of two or more chemical combinations.

The classification of urinary concretions is based on the chemical constituents of which they are composed. Before examining a calculus chemically a thorough optical examination should be made, as this may give a definite clue as to its composition. After this preliminary examination, the calculus is ground to a fine powder and examined according to the following table of Heller.

Uric Acid Calculi.

These are, perhaps, the most common renal stones. They are not always composed of pure uric acid, but are made up of a mixture of this substance with the urates. They are always colored, usually yellowish or brownish, but may at times appear distinctly red. They are fairly hard and usually show a rough irregular nodular surface, although at times this may be smooth. They fracture very easily and show, on cross section, a distinctly laminated structure, the layers frequently being of different colors, in some cases even being composed of deposits other than uric acid. If heated on a platinum foil they are combustible, burning without a flame. They give the murexid test and do not liberate appreciable amounts of ammonia on treatment with sodium hydrate.

Ammonium Urate Stones.

The ammonium urate calculi occur rarely in the adult. They are small, yellow and very soft, being distinctly clay-like and easily powdered when dry. These stones give the murexid test and also give a strong reaction for ammonia on treatment with sodium hydrate.

Calcium Oxalate Stones.

Next to the uric acid calculus the oxalate stone is most frequently met.² The smaller types of these calculi are practically colorless and have a smooth surface, while the larger ones are grayish, brownish or even black in color and have a rough nodular surface with sharp projecting angles. These stones frequently cause severe hemorrhage and much irritation when passing through the ureter and urethra. From their appearance they have been

¹ See Posner, *Ztschr. f. Urol.*, 1913, VII, 799; also, Lichtwitz, *Ibid.* 810; also, Williams, *New York Med. Jour.*, 1915, CII, 609; Woolley, *Jour. Lab. and Clin. Med.*, 1916, I, 848; Smith, *Boston Med. and Surg. Jour.*, 1917, CLXXVI, 524; Young, *Ibid.*, 1919, CLXXXI, 573; Ascanio-Rodriguez, *Rev. de Med. y Cir.*, 1919, II, 293; Hijmans, *Nederl. Tijdschr. Geneesk.*, 1919, II, 1159; Holländer, *Berl. klin. Wchnschr.*, 1919, LVI, 1129; Tardo, *Políclinico*, 1920, XXVII, 225; Schweizer, *Schweiz. med. Wchnschr.*, 1921, LI, 121; Keyes, *Am. Jour. Med. Sc.*, 1921, CLXI, 334; Cyranka, *Arch. f. klin. Chir.*, 1921, CXVI, 567.

² See Rowlands, *Biochem. Jour.*, 1908, III, 346; Mackarell, Moore and Thomas, *Ibid.*, 1910, V, 161; Kahn and Rosenbloom, *Jour. Am. Med. Assn.*, 1912, LIX, 2252; also, Kahn, *Arch. Int. Med.*, 1913, XI, 92; Kahn, *Ztschr. f. exp. Path. u. Therap.*, 1915, XVII, 88; Rosenbloom, *Jour. Am. Med. Assn.*, 1915, LXV, 161; Cabot, *Surg. Gyn. and Obs.*, 1915, XXI, 403; Braasch and Moore, *Jour. Am. Med. Assn.*, 1915, LXV, 1234.

HELLER'S TABLE FOR EXAMINATION OF URINARY CALCULI.

ON HEATING THE POWDER ON PLATINUM FOIL IT

DOES NOT BURN.

DOES BURN.

DOES NOT BURN.		DOES BURN.	
The powder when treated with hydrochloric acid.		With flame.	Without flame.
Does not effervesce.		Flame yellow and continuous. yellow, burning at the murexid test. The powder dissolves in alcohol and ether. Powder soluble in alcohol and in ammonia, cence. The powder when treated with a little potassium hydrate gives.	The powder gives the murexid test.
The gently heated powder with hydrochloric acid.		Flame pale blue, peculiar sharp der dissolves in alcohol and ether. Powder soluble der dissolves out effervesces. The powder when treated with a little potassium hydrate gives.	The powder when treated with potassium hydrate gives.
The powder when moistened with a little potassium hydrate.		Flame pale blue, peculiar sharp der dissolves in alcohol and ether. Powder soluble der dissolves out effervesces. The powder when treated with a little potassium hydrate gives.	The powder when treated with potassium hydrate gives.
Abundant ammonia. The or, at least, only powder dis-traces of it. solves in acetic Powder dis-solves in acetic acid. This so-lution gives an acid. This so-crystalline pre-lution gives an precipitate with ammoniacal ammonia.	Effervesces.	Flame pale blue, peculiar sharp der dissolves in alcohol and ether. Powder soluble der dissolves out effervesces. The powder when treated with a little potassium hydrate gives.	The powder when treated with potassium hydrate gives.
Triple phosphate mixed with an known amount of earthy phosphates.	Magnesium carbonate.	Flame pale blue, peculiar sharp der dissolves in alcohol and ether. Powder soluble der dissolves out effervesces. The powder when treated with a little potassium hydrate gives.	The powder when treated with potassium hydrate gives.

called the mulberry calculi. They are, perhaps, the hardest of the urinary stones.

These calculi are insoluble in acetic acid, but soluble in hydrochloric acid without effervescence unless the powder is previously heated. It is rare to find these stones perfectly pure, admixtures with various other sediments leading to distinct concentric arrangement as shown on fracture.

Phosphatic Calculi.

Stones composed of pure alkaline phosphates or triple phosphates are exceedingly rare. Usually the phosphatic calculi contain admixtures of ammonium urate, calcium carbonate, and calcium oxalate. The color of such stones may range from a white through yellow to some with distinct reddish tones. They are frequently of very large size, especially when formed in the bladder, are of a chalky consistency, and show a rough surface.

These calculi are soluble in hydrochloric or acetic acid, such solutions giving reactions both for phosphoric acid and the alkaline earths.

Calcium Carbonate Calculi.

Such stones are exceedingly rare. They are small in size, are distinctly chalk-like in consistency and color, and have a smooth surface. If treated with acid, carbon dioxid is evolved.

Cystin Calculi.

These stones are white or pale yellow in color, have either a smooth or irregular surface, and are soft and waxy in consistency. They vary in size occasionally being found as large as a hen's egg, although those of true renal origin are about the size of a pea. The formation of such calculi and their passage through the ureter and urethra constitute practically all of the untoward symptoms shown by subjects affected with cystinuria.¹

Such stones burn readily if heated on a platinum foil, giving off a peculiar sharp odor. The powder is soluble in ammonia from which the characteristic hexagonal plates separate on allowing the ammonia to evaporate.

Xanthin Calculi.

These stones occur especially in children, although even here they are very rare. They are usually light brown in color, moderately hard, and vary in size from that of a pea to a tennis-ball. On cross section they appear amorphous and if rubbed take a polish much resembling that of wax. The powder shows the typical reaction for xanthin previously outlined.²

¹ See Frankenthal, *Deutsch. Ztschr. f. Chir.*, 1914, CXXXI, 442; Neumann, *Deutsch. med. Wchnschr.*, 1914, XL, 2065; Aberhalden, *Schschr. f. physiol. Chem.*, 1919, CIV, 129; Mörner, *Upsala Läkaref. Förhandl.*, 1920, XXV, 265; *Ibid.*, 1921, XXVI, 1. Deniges (*Jour. soc. pharm. Bordeaux*, 1920, LVIII, 8) has introduced the following microchemical reaction for cystin in calculi: Place a small quantity of the powdered or finely shaved material on a glass slide, moisten with a drop of conc. HCl added at the outer edge by means of a pointed glass rod, and examine under the microscope without the use of a cover glass. The groups of prismatic needles are cystin hydrochlorid. After a few minutes, add a drop of water and mix. The crystals will be observed to dissolve. This is characteristic of cystin hydrochlorid and serves to distinguish it from uric acid. Evaporate the solution to dryness over a flame, cool, put, on a cover glass, add a drop of water at the edge and examine. The formation of hexagonal plates of cystin will soon be observed.

² See Rosenbloom, *New York Med. Jour.*, 1915, CI, 120.

Urostealith Calculi.

These masses consist of fat, calcium and magnesium soaps, and cholesterol. They are usually soft and may be somewhat irregular in shape. This material burns with a pale yellow flame giving an odor of resin. The dry powder is soluble in alcohol and ether, from which rhombic notched plates of cholesterol separate on evaporation.¹

VI. FUNCTIONAL DIAGNOSIS

It is usually of great importance, especially in cases in which surgical intervention is contemplated, to know just exactly what the functional capabilities of the kidney are. If one kidney is to be removed, the question arises as to whether the remaining kidney can sufficiently accommodate itself to the increased work which must be put upon it.

As such a variation has been occasionally found between the results of the chemical and microscopical examination of the urine on the one hand and the pathologic condition shown in the kidneys postmortem on the other, an attempt has been made to find delicate tests by which a true renal lesion might be indubitably determined and thus permit of an absolute diagnosis even though the urinary findings were or were not conclusive. It is granted that a more or less severe lesion of the kidney may exist and yet its functional capacity be almost normal. If this functional capacity can be determined by tests which are more or less simple, it is evident that such methods should form part of the daily routine of the practitioner.²

The methods of functional renal diagnosis fall, in a general way, into four groups.

1. The determination of the rate of excretion in the urine of a known amount of a chemical substance, injected or ingested. Especially noteworthy in this group is the method of Rowntree and Geraghty, which will be discussed in detail. Other tests, along this line, are those advanced by Schlayer and Takayasu³ of the intravenous administration of 20 c.c. of a 10 per cent. solution of lactose or the administration, per os, of 0.5 gram of potassium iodid and the determination of the time and degree of elimination. These latter tests are not, in the writer's opinion, as reliable as that of Rowntree and Geraghty and will be omitted, although they are worthy of trial in doubtful cases.

¹ Meyer and Herzog (*Med. Klin.*, 1921, XVII, 1056) report a case of albumin urinary calculi. Randall (*Jour. Urol.*, 1921, V, 119) reports a vesical calculus weighing 4 pounds.

² See Blum, *Renal Diagnosis in Medicine and Surgery*, New York, 1914; Stevens, *Jour. Am. Med. Assn.*, 1914, LXII, 1544; Geraghty, *New York Med. Jour.*, 1914, C, 312; Piersol, *Interstate Med. Jour.*, 1914, XXI, 1165; Thomas, *Jour. Am. Med. Assn.*, 1914, LXIII, 1909; Braasch and Thomas, *Ibid.*, 1915, LXIV, 104; Geraghty, *Bull. Johns Hopkins Hosp.*, 1915, XXVI, 155; Kidd, *Lancet*, 1922, I, 788; Mosenthal, *Ohio State Med. Jour.*, 1922, XVIII, 348.

³ *Deutsche Arch. f. klin. Med.*, 1911, Cl, 333; see, also, von Noorden, *Med. Klin.*, 1916, XII, 5; Treupel, *Deutsche med. Wchnschr.*, 1916, XLII, 155; Falk and Sugura, *Jour. Pharm. and Exper. Therap.*, 1917, IX, 241. Violle (*Ann. de Med.*, 1920, VII, 272; *Ibid.*, 1921, IX, 330) advocates the "hippuric acid test" as very reliable. After the elimination of hippuric acid is determined on a definite diet, the patient is given two doses of 0.5 gram each of benzoic acid and glyccoll, the elimination of the synthesized hippuric acid being then determined during the day of the test.

2. The comparison of the excretion of substances normally eliminated, as water,¹ salt, and nitrogenous bodies, with the amount of intake of such substances in a test diet. This method has been especially elaborated by Mosenthal and will be freely discussed. Similar, although by no means as reliable, are the urea² and salt excretion tests, following the administration of a known quantity of these substances.

3. The determination of the extent of retention in the blood of normal metabolic products, especially of nitrogenous substances. This phase of the renal tests will be discussed in detail in the section on Blood.

4. A determination of the ratio between the concentration of urea in the blood and its excretion in the urine. The results are expressed in terms of Ambard's coefficient or of McLean's Index. These will be taken up in the section on Blood.

It is to be mentioned that no one test of renal function is to be regarded as a standard for all cases. So many factors enter into the consideration of this question, that "to speak of testing renal function as a whole is to set a false standard." It is only when all the available data is before one, that proper deductions may be made. This has been excellently expressed by Mosenthal and Lewis in the following words: "Of the various tests for renal function, each has its own significance, and a greater insight will be obtained into the characteristics of kidney disease when physicians will no longer advocate one test blindly to the exclusion of all others, but will endeavor to interpret each one according to its significance."

Cryoscopy.

The method of determining the freezing-point of a solution is one of the most delicate of those of physical chemistry. As it is based upon the principle that substances in solution lower the freezing-point of the solvent in direct proportion to the molecular or ionic concentration of the solution, this method serves as a ready means of determining the molecular weight of a substance as well as the molecular concentration of a solution. For a successful outcome of a cryoscopic determination, the most assiduous attention to detail must be paid, so that this method certainly can find no place in the hands of the general practitioner or even in those of the laboratory worker, who has not been especially trained along these lines. Such being the case, it is absurd to expect that slight variations in the freezing-point (Δ) of such a complex mixture as the urine can yield any valuable information, especially when one remembers that fluctuations wider than those shown under pathological conditions may be noted as a result of not observing such slight details as that of constantly agitating the urine during the cooling and freezing. The normal freezing-point of the urine varies between -0.9 and -2°C. , an increase being known as hypersthenuria and a decrease as hyposthenuria.

¹ See Lehmann and Elfeldt, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1921, XXXIV, 291; Richards, *Am. Jour. Med. Sc.*, 1922, CLXIII, 1.

² Higley and Upham (*Arch. Int. Med.*, 1920, XXVI, 367) have studied the renal concentration power for uric acid and Magath (*Jour. Lab. & Clin. Med.*, 1921, VI, 463) has introduced a method for renal insufficiency based upon the provocative uric acid concentration.

As will be seen in the discussion of this subject in the section on Blood, the results obtained by this method have been far from satisfactory, as nothing of distinct diagnostic value has as yet been derived from comparative studies by various workers.¹ It would seem to the writer, therefore, that for the present this method would better be left to the research worker than to be adopted by the general or special student, who should make use of methods which will yield results of more immediate value to him. For these reasons the writer must refer to other works for a detailed description of the method.

Electric Conductivity.

The remarks made later in the section on Blood regarding electric conductivity are especially applicable to the urine. This test is altogether too delicate to be applied to such complex fluids as the urine with the hope that slight variations in the conductivity will show anything of importance. As the electric current is conducted only by dissociable compounds, this method can show absolutely nothing regarding the excretion of the nondissociable organic substances. Such being the case the writer can see no reason for resorting to such delicate procedures as the determination of the conductivity of the urine when no attempt is made, as may be observed in many of the experiments reported, to control the intake of the inorganic substances which would affect the conductivity of the urine. A little more attention to ordinary methods of chemical examination, with especial regard to ascertaining the intake and output of the patient, would, in the writer's opinion, yield much more valuable information than could be obtained by the rather uncertain urinary manipulations with the method of Kohlrausch.

Mosenthal's Test Meal for Renal Function.

In 1914 Hedinger and Schlayer² proposed a test for estimating renal function based upon the response of the kidney, as shown by its output, to an intake of a dietary containing considerable quantities of diuretic substances such as fluids, salts, and purins. The various influences, which sodium chlorid, for instance, may exert upon the excretion of water had been previously shown by Mosenthal and Schlayer.³ Before administering their test diet, Hedinger and Schlayer placed the subject for the three days preceding the test upon a reasonable general diet, which contained, as necessary constituents, 8 to 12 grams of salt and about 2000 c.c. of water. If this amount of fluid was deemed too large, a smaller amount might be used but the amount on each of the three days should be the same. This preliminary attention to diet is deemed by these authors of importance in establishing a definite fixed output of water and salt. On the day of the test, the patient is directed to empty his bladder completely at 7 A.M. and is then started on his "test meal," which is divided into 5 meals at different hours throughout the day. The urine is collected at two-hour intervals until 9 P.M., when the total voiding from that hour until 7 A.M. is collected in one container. In

¹ See Desgrez, *Bull. soc. pharmakolog.*, 1915, XXII, 159.

² *Deutsch. Arch. f. klin. Med.*, 1914, CXIV, 120.

³ *Ibid.*, 1913, CXI, 217.

each specimen of the day urine and in the total night urine the specific gravity, total volume and amount of NaCl are determined. O'Hare¹ has followed this method in a study of the renal function in nephritis.

In 1915 Mosenthal² introduced a modification of this method, which, with the further changes suggested by him in 1918, is the method followed in performing this very valuable and important test. The directions for this test meal are taken from his original article and comprise the instructions given to the nurse in charge of the case.

All food is to be salt free food from the diet kitchen.

Salt for each meal will be furnished in weighed amounts. (One capsule of salt, containing 2.3 grams of sodium chlorid, is furnished for each meal. The salt which is not consumed is returned to the laboratory, where it is weighed and the actual amount of salt taken is calculated.)

All food or fluid not taken must be weighed or measured after meals and charted in the spaces below.

Allow no food or fluid of any kind except at meal times.

Note any mishaps or irregularities that occur in giving the diet or collecting the specimens.

Breakfast, 8 A.M.

Boiled oatmeal, 100 gm.
Sugar, 1-2 teaspoonfuls
Milk, 30 c.c.
Two slices bread (30 gms. each)
Butter, 20 gms.
Coffee, 160 c.c.
Milk, 40 c.c.
Sugar, 1 teaspoonful
Milk, 200 c.c.
Water, 200 c.c.

Dinner, 12 Noon.

Meat soup, 180 c.c.
Beefsteak, 100 gms.
Potato (baked, mashed or boiled), 130 gms.
Green Vegetables, as desired
Two slices bread (30 gms. each)
Butter, 20 gms.
Tea, 180 c.c.
Sugar, 1 teaspoonful
Milk, 20 c.c.
Water, 250 c.c.
Pudding (tapioca or rice) 110 gms.

Supper, 5 P.M.

Two eggs, cooked in any style
Two slices bread (30 gms. each)
Butter, 20 gms.
Tea, 180 c.c.
Sugar, 1 teaspoonful
Milk, 20 c.c.
Fruit (stewed or fresh) 1 portion
Water, 300 c.c.

The patient empties his bladder at 8 A.M. (the beginning of the test meal), the specimen being discarded. The urine is collected in separate containers at two-hour intervals until 8 P.M. The total voiding from 8 P.M. to 8 A.M. is collected in one container and constitutes the night urine.

¹ Arch. Int. Med., 1916, XVII, 711.

² Ibid., 1915, XVI, 733.

This dietary contains approximately 13.4 grams of nitrogen, 8.5 grams of salt, 1760 c.c. of fluid and a considerable quantity of purin material in the meat, soup, tea, and coffee. All these substances act as diuretics, and it is on the mode of excretory response to such stimuli that the test of renal function depends.

In the earlier studies with this test, the above dietary was followed, but it has become questionable whether such a rigid adherence to it is necessary, as such practice makes it difficult to follow in private work. In his later article Mosenthal¹ has studied this test under the influence of 3 different dietaries, namely, the above, which he calls his "high protein diet;" a "low protein diet," which is the usual one consisting of some such combination as the following:

Breakfast.

Sherry (30 c.c.), baked apple, stewed prunes, orange, hominy cornstarch ($\frac{2}{3}$ hominy, $\frac{1}{3}$ cornstarch), cereal, cream (15 c.c.), sugar, butter.

Dinner.

Sherry (30 c.c.), potato (baked or mashed), string-beans, cabbage, carrots, lettuce, onions, tomatoes, cucumber pickles, fruit cornstarch pudding, tapioca pudding, sugar, butter. .

Supper.

Same as dinner.

No restrictions being placed on the intake of this diet, the nitrogen equivalent is about 3 to 4 grams. The third diet studied by Mosenthal was styled the "normal diet," which consisted of the food which the patient chose for himself. Specimens were collected as in the test with the "high protein diet," outlined above, the only exception being that if the night meal was later than 5 P.M. the collection of the night specimen began three hours after this meal. The results of these studies is that the response of the kidney is not dependent, as far as the interpretation of this test is concerned, on the diet, so that the value of the test is widely extended to general private practice.

As in the original test of Hedinger and Schlayer, the volume and specific gravity of each specimen collected are determined. The salt and nitrogen may be determined in each specimen, but all that is necessary is to make such determinations in the total day and total night urines.

In the earlier work with this test, the standards set as normal have been shown to be, to a certain extent at least, erroneous, chiefly owing to neglect in considering the extra-renal factors which influence the output of the kidney. "Nervous tension, very hot or cold weather, and what may be termed the 'renal habits' of any person, all have appeared to play a distinct rôle in causing differences to appear that were not at first appreciated." To these conditions we must add the effect of the state of the water reserve of the

¹ Arch. Int. Med., 1918, XXII, 770. See, also, Mosenthal and Lewis, Jour. A. M. A., 1916, LXVII, 933; Desha, Southern Med. Jour., 1916, IX, 1041; Christie, Jour. Lab. and Clin. Med., 1917, II, 899; Moses, Med. Record, 1917, XCI, 273; Vaughan, Jour. Lab. and Clin. Med., 1918, III, 531; Veer and Saunders, Jour. A. M. A., 1919, LXXII, 1586. Sharlit and Lyle (1921, XXVIII, 640) believe the estimation of total solids to be a better means of interpretation than the specific gravity.

tissues and the chilling of the body surface, as shown by Lyle and Sharit.¹ These authors state "These factors can function significantly to distort the test meal reaction when viewed in the terms suggested by Mosenthal. When so functioning they affect the fluid element in the test meal reaction; and this mainly by virtue of the fact that the skin and lungs make a preferential and significant demand on body fluids, whereas the excretion of solids by the skin and lungs is practically negligible."

The normal standard, as originally advocated by Mosenthal, was the following: A maximum specific gravity of 1018 or higher; a variation of 9 degrees or more between the minimum and maximum specific gravity readings; and a night urine of 400 c.c. or less, with a specific gravity of 1018 or over and a concentration of nitrogen of at least 1 per cent.; the balance between the output and intake of salt, nitrogen, and fluids should be approximately equal. For the reasons mentioned in the previous paragraph, these standards can no longer be regarded as correct. The newer standards, as advanced by Mosenthal, represent the present conceptions of the renal response to this test, the chief changes being noted in the characteristics of the night urine. Under the influence of a high or a low protein diet or of the normal diet, the maximum specific gravity is to be regarded as 1018 with the high diet and 1020 with the low or normal diets. A variation of 9 points between the high and low specific gravity should obtain. With the normal diet, the degrees of variation in the specific gravity readings is of no value. The specific gravity of the night urine has no significance in this new standard. The volume of the night urine should be 750 c.c. or less. The nitrogen and sodium chlorid in the night urine or the highest per cent. in any specimen is regarded as normal if 1 per cent. or higher, but as not necessarily abnormal, if less.

When kidney function becomes involved, the first signs are usually demonstrated in the night urine, the quantity increasing. While the specific gravity and nitrogen concentration may become lowered, yet this is not necessarily true, so that these factors are no longer regarded as important. Marked variations may be brought about in the elimination of edema fluids, so that the change from oliguria to polyuria may appear with rapidity and influence the interpretation of this test. In severe cases of nephritis, an advanced degree of functional inadequacy of the kidney is indicated by a markedly fixed and low specific gravity, a diminished output of both salt and nitrogen, a tendency to total polyuria and a night urine showing an increased volume, with, usually, a low specific gravity and low concentration of nitrogen. As Mosenthal states, these functional pictures are found regularly in many other conditions, as pyelitis, cystitis, hypertrophied prostate, marked anemia,² pyelonephritis, polycystic kidney and diabetes insipidus. In chronic diffuse nephritis, the condition of renal function is characterized by its variability. The findings in myocardial insufficiency vary according to the activity of the heart. This test gives the earliest indication of dim-

¹ Arch. Int. Med., 1918, XXI, 366.

² Christian, *Ibid.*, 1916, XVIII, 429.

inished kidney efficiency and, likewise, reaches the maximum degree of impairment before the other functional tests. A careful study of this is recommended.

Phloridzin Test.

This test is based on the assumption that phloridzin normally gives rise to a glycosuria through distinct alternations in the renal cells. In other words, a so-called "renal diabetes" is set up. The technic is as follows: 1 c.c. of a 1:200 aqueous solution of phloridzin is injected subcutaneously. The urine is tested at intervals of 15 minutes for the appearance of sugar. Normally, sugar may be detected in one-half to one hour and may be present for as long as five hours. The quantity eliminated may vary from 0.5 to 3 grams of glucose. In nephritis the sugar is usually absent or below 0.5 gram. The test does not distinguish the various types of nephritis, yet it does usually indicate that renal activity is disturbed. It is, however, being rapidly displaced by the following test.

Phenolsulphonephthalein Test.

Rowntree and Geraghty¹ have introduced a test for the functional activity of the kidney which is one of the most accurate at our disposal.² The solution used is prepared as follows: 0.6 gram of phenolsulphonephthalein and 0.84 c.c. of 2/N NaOH solution (8 per cent.) are added to sufficient 0.75 per cent. NaCl solution to make 100 c.c. Each c.c. of this solution contains, therefore, 6 mg. of the dye. As the mono-sodium salt, formed in this solution, is slightly irritant locally when injected, 2 or 3 drops more of the 2/N NaOH are added, when the solution changes to a beautiful Bordeaux red color and becomes non-irritant.³

The technic is as follows: 20 to 30 minutes before applying the test, the patient is given 300 to 400 c.c. of water in order to insure a free urinary secretion, otherwise delayed time of appearance may be due to lack of secretion. Under aseptic precautions a catheter is introduced into the bladder and the bladder completely emptied. Noting the time, 1 c.c. of the above solution is administered subcutaneously in the upper arm by means of an accurately graduated syringe. The urine is allowed to drain through the catheter into a test-tube in which has been placed a drop of 25 per cent. NaOH solution and the time of the first pinkish tinge is noted. In patients without urinary obstruction, the catheter is withdrawn at the time of appearance of the drug in the urine and the patient is instructed to void into a receptacle at the end of one hour and into a second receiver at the end of the second hour. A rough estimate of the time of appearance may be made by having the patient void

¹ Jour. Pharm. and Exper. Therap., 1910, I, 579; Arch. Int. Med., 1912, IX, 284.

² See Conzen, Deutsch. Arch. f. klin. Med., 1912, CVIII, 353; Smith, Am. Jour. Dis. Child., 1913, V, 25; Pepper and Austin, Am. Jour. Med. Sc., 1913, CXLV, 254; also, Rowntree, Fitz and Geraghty, Arch. Int. Med., 1913, XI, 121; Geraghty, Rowntree and Cary, Am. Surg., 1913, LVIII, 800; Dietsch, Ztschr. f. Exper. Pathol. u. Therap., 1913, XIV, 512; Rowntree, Geraghty and Marshall, Surg. Gynec. and Obs., 1914, XVIII, 196; Tardo, Jour. d'Urol. 1922, XIII, 167; Macht, Jour. Urol., 1922, VII, 273.

³ This mono-sodium salt of phenolsulphonephthalein may be obtained from Hynson, Wescott and Co. of Baltimore in the form of ampules, each c.c. of the solution containing 6 mg.

urine without the use of the catheter at frequent intervals. In prostate cases it is wise to have the catheter in place until the end of the observation. The catheter is corked at the time of appearance of the drug in the urine and the cork is removed at the end of the first and second hours, the bladder being drained each time.

Each sample of urine is measured and the specific gravity taken. Sufficient 25 per cent. NaOH is added to make the urine decidedly alkaline in order to elicit the maximum color (a brilliant purple-red). This solution is now placed in a liter volumetric flask and distilled water added to the 1 L. mark. Thoroughly mix the solution and filter a small portion for comparison with the standard solution. This standard consists of 3 mg. of phenolsulphone phthalein (or 1 c.c. of the solution used for injection) diluted to 1 liter and made alkaline with 1 or 2 drops of 25 per cent. NaOH solution. This solution will retain its intensity of color for weeks. The comparison of color is made in the Duboscq or the Rowntree and Geraghty modification of the Autenrieth-Königsberger colorimeter. The known (standard) solution is adjusted to the 10 mm. mark and the intensity of the unknown solution is made to correspond by means of the usual manipulations. One may readily calculate the amount of drug eliminated as follows: If, for instance, the reading for the unknown solution is 20, it is evident that only 50 per cent. as much dye is present as is contained in the standard solution. That is, the excretion equals 50 per cent. of the 3 mg. of the standard or 25 per cent. of the 6 mg. injected. The readings may be then made on a second and third voiding until the drug is completely eliminated.¹

In normal cases it has been found that the time of appearance varied from 5 to 11 minutes and that 40 to 60 per cent. of the drug was excreted in the first hour and 20 to 25 per cent. in the second hour. The excretion of the drug does not run parallel to the excretion of water. The smaller the amount of urine in normal cases the greater the concentration of the drug. It is immaterial, as far as the excretion of the drug is concerned, whether the urinary output is 50,200,400 or more c.c.

In pathological cases it has been demonstrated that the permeability of the kidney for this drug is decreased in both chronic parenchymatous and chronic interstitial nephritis, the decrease being most marked in the latter and varying with the intensity of the disease. The work of MacNider² would seem to indicate that the tubular epithelium is more important as a functional unit than is the glomerulus of the kidney. He shows that, in cases in which chronic injury to the kidney is chiefly evident in the glomerulus with histologically demonstrated preservation of the tubules, the phenolsulphone-

¹ In some cases the color of the urine, especially if this contains bile or blood, may interfere to some extent with the accuracy of the color comparisons. Under such conditions Burwell and Jones (Jour. Am. Med. Assoc., 1921, LXXVII, 462) recommend treatment of the urine, after it is made up to volume with a saturated alcoholic solution of zinc acetate. To c.c. of the diluted urine are added 20 c.c. of the zinc acetate solution and the mixture is filtered. Twenty cubic centimeters of this filtrate are made alkaline with 5 c.c. of saturated NaOH solution and made up to 40 with water. The comparison of colors may then be made in the usual way, the result being, of course, multiplied by 2.

² Arch. Int. Med., 1920, XXVI, 1.

phthalein excretion may be only slightly reduced and that there may be little or no retention of the blood nitrogen elements. On the other hand, when an acute tubular injury is added to the glomerular involvement, a rapid reduction in elimination of the dye and a retention of nitrogen in the blood occurs. The pigment excretion test would seem to be of greater value than the retention tests in cases in which the involvement is more especially evident in the glomeruli. According to MacNider, the injury of the kidney, as indicated by the capacity to eliminate phenolsulphonephthalein, must be of severe type before kidney function becomes sufficiently impaired to indicate the injury by a retention of urea and other nitrogen products. In cases with obstruction in the lower urinary tract, this test may show renal involvement to such an extent that operation should be deferred, especially if the time of appearance is delayed beyond 25 minutes and the output of the drug is below 20 per cent. for the first hour. This point is of special value to the surgeon as it shows the danger of using surgical intervention until the kidney may become less insufficient. It would seem to be of value, also, in the study of the renal condition preceding uremia. To the obstetrician it may show the possible approach of eclampsia, as has been demonstrated in several cases. In unilateral and bilateral kidney diseases the absolute amount of work done by each kidney as well as the relative proportion can be determined by resort to ureteral catheterization followed by this test. The writer has no hesitancy in advising a careful study of this test in all cases with suspected or actual renal involvement.²

Urea Concentration Test.

While certain phases of the urea excretion and retention will be fully

² See Behrenroth and Frank, *Ztschr. f. exper. Path. u. Therap.*, 1913, XIII, 72; Rowntree, and Fitz, *Arch. Int. Med.*, 1913, XI, 258; Geraghty, *Jour. Am. Med. Assn.*, 1913, LX, 191; Goodman, *Ibid.*, 1913, LXI, 184; Frothingham, Fitz, Folin and Denis, *Arch. Int. Med.*, 1913, XII, 245; Geraghty and Rowntree, *Jour. Am. Med. Assn.*, 1913, LXI, 939; Foster, *Arch. Int. Med.*, 1913, XII, 452; Fishbein, *Jour. Am. Med. Assn.*, 1913, LXI, 1368; Roth, *Berl. klin. Wchnschr.*, 1913, L, 1609; Marogna, *Gazz. d. osp.*, 1914, XXXV, 172; Widale Weill and Radot, *Presse méd.*, 1914, XXII, 565; Pezzana, *Policlinico*, 1914, XXI, 563; Machwitz, Rosenberg and Tschirtkoff, *Münch. med. Wchnschr.*, 1914, LXI, 1268; Hess, *Ibid.*, 1835 and 1874; Ware, *New York Med. Jour.*, 1914, XCIX, 416; Robertson, *Ibid.*, 972; Jones, *Ibid.*, 1914, C, 518; Fanz, *Ibid.*, 1214; Tracy, *Surg., Gyn. and Obs.*, 1914, XIX, 734; Block, *Jour. Am. Med. Assn.*, 1914, LXII, 1309; Griessmann, *Deutsch. Arch. f. klin. Med.*, 1914, CXIV, 32; Deutsch and Schmuckler, *Ibid.*, 61; Heding and Schlayer, *Ibid.*, 120; Hessel, *Ibid.*, 396; Lichtwitz and Stromeyer, *Ibid.*, 1914, CXVI, 127; Rowntree, *Am. Jour. Med. Sc.*, 1914, CXLVII, 352; Fitz, *Ibid.*, 1914, CXLVIII, 330; Thayer and Snowden, *Ibid.*, 781; Agnew, *Arch. Int. Med.*, 1914, XIII, 485; Frothingham and Smillie, *Ibid.*, 1914, XIV, 514; Quimby and Fitz, *Ibid.*, 1915, XV, 303; Miller and Cabot, *Ibid.*, 369; Rowntree, Marshall and Baetjer, *Ibid.*, 543; Hopkins and Jones, *Ibid.*, 964; Bauer and Habetin, *Ztschr. f. Urol.*, 1914, VIII, 355; Bauer and Nyiri, *Ibid.*, 1915, IX, 81; Tileston and Comfort, *Am. Jour. Dis. Child.*, 1915, X, 278; Hess, *Bull. Johns Hopkins Hosp.*, 1915, XXVI, 52; Smith, *Jour. Am. Med. Assn.*, 1915, LXIV, 223; Elliott, *Ibid.*, 1885; Pedersen, *New York Med. Jour.*, 1915, CI, 770; Siegel, *Pub. Jefferson Med. Coll.*, 1915, VI, 78; Hempelmann, *Am. Jour. Dis. Child.*, 1915, X, 418; Naroditzky, *Russk. Vrach.*, 1915, XIV, 850; Lubs and Acree, *Jour. Am. Chem. Soc.*, 1916, XXXVIII, 2772; Barker and Smith, *Am. Jour. Med. Sc.*, 1916, CLT, 44; Brown and Cummins, *Jour. A. M. A.*, 1916, LXVI, 793; Peabody, *Boston Med. and Surg. Jour.*, 1916, CLXXV, 158; Cameron, *Jour. A. M. A.*, 1916, LXVI, 1765; Kendall, *Ibid.*, 1917, LXVIII, 343; Arbuthnot, Snowden and Brooks, *Jour. Pharm., and Exp. Therap.*, 1917, IX, 349; Davis and Engman, *Jour. Cut. Dis.*, 1919 XXXVII, 772; Fitz, *Boston Med. & Surg. Jour.*, 1920, CLXXXIII, 247; Pedersen, *New York Med. Jour.*, 1920, CXII, 477; Braasch and Kendall, *Jour. Urol.*, 1921, V, 127; Tardo, *Jour. d' Urol.*, 1921, XII, 393; Snowden, *Arch. Int. Med.*, 1921, XXVIII, 603; Peterson, *Jour. Urol.*, 1922, VII, 73.

discussed in the Section on Blood, this new test of functional renal activity properly falls in the present chapter.

MacLean and de Wesselow,¹ starting with the idea that urea is one of the earliest and most readily retained substances in involvement of kidney function, have originated the following provocative urea concentration test. The patient is instructed to empty his bladder completely and is then given, by mouth, 15 grams of urea dissolved in 100 c.c. of water and flavored with tincture of orange. The patient passes urine at the end of one hour and at the end of two hours, both specimens being measured and saved for analysis. The administration of the urea induces in some individuals a diuresis, so that should the hourly specimen measure more than 150 c.c. a third hourly specimen is collected. Urea is estimated by the hypobromite method. From their results in a study of 1200 cases, the authors conclude that a urea concentration of 2 per cent. means a fairly efficient kidney and less than a per cent. a diseased kidney, the lower the concentration the more serious the kidney damage. MacLean and Russel later advise saving only the second hour specimen for the determination of urea, but state that the first should be measured in order to note any diuretic effects.

Weiss, in his work with this test, determined the urea concentration of the urine for the two hour period prior to the administration of the urea and, also, the concentration of the second hour specimen after the intake of urea. In addition, the blood urea was, also, determined both before and after a phthalein test was performed at the same time. His figures indicate that the urea concentration for the second hour closely approximates the phenol-sulphonephthalein elimination. Normal individuals concentrate at about 3 per cent.; in cases of mild chronic nephritis the concentration falls to 1.75 per cent., severe chronic nephritis to 1.3 per cent., and severe chronic cases to 0.8 per cent. Harrison finds this test very useful as an indicator of renal activity and calls attention to the following points: (1) it is advisable to withhold fluids for at least six hours before the test; (2) it is safest to measure the volume of urine passed each hour, a volume greater than 150 c.c. for first hour or 100 c.c. second and following hours generally indicating diuresis when a result below 2 does not necessarily mean an inefficient kidney; (3) 15 grams of urea may not always be sufficient to provoke a concentration greater than 2 per cent. although the kidneys are efficient; (4) it is wise to check the urine findings by a blood urea determination. Although this test is of special value in chronic nephritis, it is interesting to note, from the study of a case of severe acute nephritis following mercuric chlorid poisoning by Funk and Weiss, that the administration of urea was well tolerated and showed the same lack of concentration as noted in chronic cases.

¹ Brit. Jour. Exp. Path., 1920, I, 53. See, also, Weiss, Jour. Am. Med. Assoc., 1921, LXXVI, 298; Silvertown, Med. Jour. Australia, 1921, I, 397; Walker, Lancet, 1921, I, 1126; Rabinowitch, Arch. Int. Med., 1921, XXVIII, 827; Harrison, Brit. Jour. Exp. Path., 1922, III, 28; Funk and Weiss, Jour. Lab. & Clin. Med., 1922, VII, 233. Black, Am. Jour. Med. Sc., 1922, CLXIII, 218; Weiss, Penn. Med. Jour., 1922, XXV, 607.

CHAPTER VII

SECRECTIONS OF THE GENITAL ORGANS

I. MALE SECRECTIONS

General Considerations.

The normal secretion of the male generative organs is known as semen and is a mixture of the secretions of the prostate gland, the glands of Cowper, the testicles, and the seminal vesicles. It is a practical impossibility from the clinical standpoint to separate the different elements of the semen, so that this must be discussed as a whole.

The prostatic fluid is often obtained for diagnostic examination. For this purpose the prostate is "milked" by the finger in the rectum. After washing out the urethra, the finger is passed into the rectum and the prostate massaged vigorously. The fluid soon begins to appear at the meatus as a grayish-white, yellow-greenish, turbid fluid, which may be distinctly purulent or bloody. It is tenacious and somewhat sticky, and has a low specific gravity. It is, perhaps, most frequently examined bacteriologically, especially for gonococci.

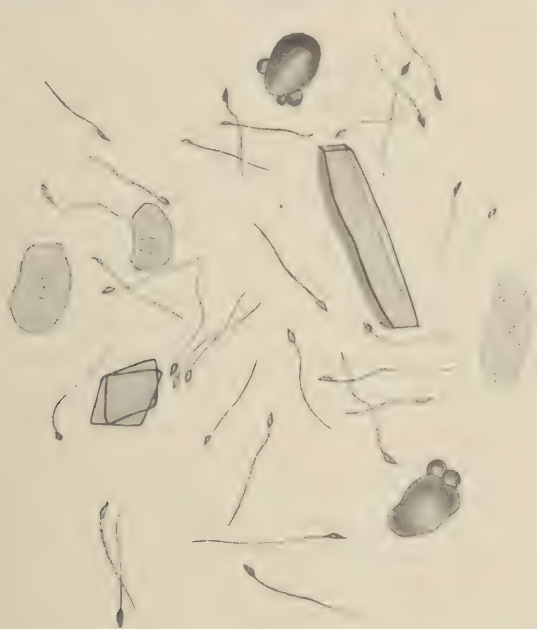
The semen is a white or slightly yellowish, somewhat thick and viscid liquid with a peculiar odor, somewhat resembling fresh glue, and showing a neutral or faintly alkaline reaction, a nonhomogeneous milky appearance, and a specific gravity greater than that of water. It is composed of semisolid material in the form of white masses floating in a limpid liquid and holding in suspension specific elements, derived from the secretory glands of the genital apparatus and from the desquamation of the various canals through which the semen passes.

Semen contains about 6 per cent. of organic and 4 per cent. of inorganic matter. Its chief chemical characteristic is the presence of spermin (C_2H_5-N)₂, which is at least isomeric, if not identical, with diethylen-diamin, according to Ladenburg and Abel. This spermin, which is derived largely from the prostate gland, combines with the phosphoric acid radical to form spermin phosphate, which crystallizes in the form of four-sided spindles or prisms which may appear as flattened needles. In some cases these crystals resemble very closely the diamond-shaped double pyramids known as Charcot-Leyden crystals, which are found in the sputum. They are, however, of a different crystalline group and are soluble in formalin, while those found in the sputum are insoluble in this menstruum. These spermin crystals are known as Böttcher's crystals. Miescher has studied the composition of the heads of the spermatozoa and has been able to isolate certain bodies, known as protamins, which are the simplest type of protein material.¹

¹ See Steudel, *Ztschr. f. physiol.Chem.*, 1913, LXXXIII, 72.

Microscopic Examination.

The most important and characteristic constituent of semen are the spermatozoa.¹ These sexual elements consist of an anterior broader portion or head and a narrow thread-like tail. The former is oval or egg-shaped and measures about 5 microns in length, 4 in breadth, and 2 in thickness. Just behind this pyriform head is a short cylindrical portion measuring 6 microns in length, which is known as the middle piece. This tapers somewhat to the



Katharine Hill.

FIG. 115.—Normal semen.

point of union with the tail. This so-called tail is a thread-like posterior portion and is approximately 45 microns in length. In the freshly voided semen these tail portions show active undulatory whip-like motions, which persist for 24 to 48 hours, and even longer under proper conditions,² and enable the spermatozoa to progress from point to point. Alkalies seem to favor this movement, while dilute acids inhibit it very rapidly. This movement³ of the spermatozoa is closely associated with their sexual activity, as the cells showing no movement when freshly voided may be regarded as possessing no

¹ Koessler (Trans. Chic. Path. Soc., 1912, VIII, 280) has shown that the spermatozoa may act as direct carriers of infectious agents, among these being the *spirochaeta pallida*. Widakowich (Semana Med., 1920, XXVII, 633; Rev. de la Asoc. Méd. Argentina, 1921, XXXIV, 1504) calls attention to the fact that deformed and anomalous spermatozoa are especially prevalent in syphilitics.

² See Ochi, Acta Scholæ Med. Univ. Imp. in Kioto, 1916, I, 341; Sato, Ibid., 361.

³ The statement is frequently made that spermatozoa are motionless till they get beyond the epididymis, but Hühner (Jour. A. M. A., 1917, LXVIII, 1340) calls attention to the motility of the spermatozoa on the testicular side of the epididymis.

functional power. For a discussion of spermatogenesis as well as of fertilization of the ovum the writer would refer to the admirable description of McMurrich.¹

Besides these characteristic portions of the semen, large numbers of lecithin globules are seen, which give the milky appearance to the fluid. Especially to be noted among the cellular elements are the so-called corpora amylacea which resemble very closely starch granules, having concentric striations, a finely granular center and occasionally a nucleus. These cells take a distinct blue color on treatment with iodine solution.² Moreover, various epithelial cells are observed, which are derived from the several glands contributing to the composition of the semen. Some of these cells are distinctly granular, some contain fat globules and very closely resemble the colostrum corpuscles of the lacteal secretion, while some of the granules resemble myelin. In rare cases cylindrical casts are seen which simulate the hyaline casts of the urine, but as a rule they are larger and longer. These are supposed to be derived from the prostate gland and seminal vesicles. If the semen be allowed to stand for a few minutes, several types of crystal may be observed, especially the phosphate of spermin, ammonium-magnesium phosphate, fatty acids, and oxalate of calcium. This last crystalline component is especially noted in the urine in cases of spermatorrhea.

Pathologic Variations.

Direct pathologic variations in the semen are limited to two conditions. Either spermatozoa are absent or those present are nonmotile. The determination of the presence of spermatozoa in the semen or in suspected stains is a matter of simple microscopic examination. With the question of the motility of such elements, when present, the conditions under which the examination is made may markedly influence the findings. If possible, semen should be examined, with regard to the motility of the spermatozoa, as soon as ejected, but if such is not possible the fluid must be kept warm until examination may be made. It is absolutely out of the question to make a positive diagnosis of true nonmotility of spermatozoa from examination of specimens, which have been allowed to cool. In some cases, if the time has not been too extended, warming may bring back the motile power of these cells, but in many cases it does not. It is, therefore, unjust and unwise to brand a man as sterile without absolute proof that such a condition really exists. If no spermatozoa are present, especially after several examinations, sterility is absolute. This condition is known as azoöspERMATISM. According to Kehrer, 40 per cent. of cases of conjugal sterility are due to the absence of spermatozoa in the semen. It is, therefore, necessary that the ordinary gynecological idea that women are the responsible factors in the family sterility should be, at least, partly borne by the man, as it is unjust to the woman to blame her for faults existing in the husband. The writer does not wish to be interpreted as stating that sterility does not frequently exist in

¹ *The Development of the Human Body*, Philadelphia, 1907.

² Fischer (Ill. Med. Jour., 1922, XLI, 106) believes that these bodies are really starch grains arising from the condom in which the specimens are usually submitted for examination.

women, but he does desire to emphasize the point that many more men are sterile than is generally supposed and that the sterile women are in this condition largely through the results of gonorrheal infection through their husbands.¹

Spermatozoa may be absent from the semen during convalescence from acute febrile conditions, valvular heart disease, and in general conditions associated with lowered nutrition. On the other hand, the constant presence of spermatozoa in the urine, as well as in the semen, may be noted as a result of various pathological conditions as well as of venereal excesses or masturbation. To this condition is given the name of spermatorrhea.

Medicolegal Aspects.

Not infrequently the physician is called upon to decide whether certain stains are due to spermatic fluid or whether assault has been committed. If the question is one of suspected rape, an examination of a drop of the vaginal fluid or of scrapings from the vulva or vagina will usually reveal the spermatozoa. Of course other signs, which are important from the medicolegal point of view, will be observed in the examination of the external organs.

The stains usually subjected to medicolegal examinations for the presence of spermatic fluid have a grayish-yellow color, their size is somewhat variable, their contour usually irregular, and the linen upon which the stain is usually found is almost as stiff as if it had been starched. As spermatozoa are very resistant to the action of reagents as well as to putrefactive processes, they may be detected many years after the stain was made. It is, therefore, almost an impossibility to say by examination of a stain anything about the length of time the stain has been upon the cloth.

A fragment of the linen, which shows the stain, is placed in a watchglass and allowed to soak for one hour in 30 per cent. alcohol or in faintly alkaline water.² It is then lifted from the solvent, placed in another watchglass, and teased with needles in a solution of 1 per cent. eosin in glycerin. A few drops of this mixture are then placed upon a glass slide, covered with a cover-glass and examined with a high-power dry lens. Spermatozoa, if present, will practically always be seen by this method. The heads are stained a deep red while the tails, which are usually broken off by the teasing, show a light reddish tint, which distinguishes them from the unstained vegetable fibers.

Florence's Test.³

It not infrequently happens that spermatozoa may not be found, although the stain be due to spermatic fluid. The principle of this test is that spermatic fluid when treated with a solution of iodine in potassium iodide gives

¹ See Reynolds, *Jour. Am. Med. Assn.*, 1913, LXI, 1363; *Ibid.*, 1915, LXV, 1151; Dozzi, *Gazz. d. osp.*, 1914, XXXV, 2569; Polak, *Surg. Gyn. and Obs.*, 1916, XXIII, 261; Reynolds, *Jour. A. M. A.*, 1916, LXVII, 1193; Lespinasse, *Ibid.*, 1917, LXVIII, 345; Reynolds, *Ibid.*, 1919, LXXIII, 1099. Reynolds and Morcomber (*Jour. Am. Med. Assoc.*, 1921, LXXVII, 169) believe that defective diet plays a large role in the causation of sterility. See Henderson and Amos, *Jour. Am. Med. Assoc.*, 1922, LXXVIII, 1791.

² If the stain be relatively fresh, the bit of cloth may be cut out, placed in a watch glass and treated with dilute salt solution for two or three hours, the specimen being covered with a beaker to prevent evaporation of fluid and to protect it from dust. A drop of the turbid fluid is then examined under the microscope. Roussin advises the use of a solvent and stain consisting of 1 part of iodine, 4 parts of potassium iodide and 100 parts of water.

³ *Arch. d'Anthropol.*, 1896, X, 417 and 520; *Ibid.*, XI, 37, 146 and 249.

crystals which were supposed to be iodospermin. According to Bocarius, this substance is not iodospermin, but an iodine compound of cholin. This test would be given, therefore, by any substance containing cholin and cannot, for this reason, be distinctive for spermatic fluid. Such being the case, a negative result is of far greater importance than is a positive one, although in old stains spermatozoa may be found along with a negative Florence reaction.

Technic.

The reagent used consists of 2.54 grams of iodine and 1.65 grams of potassium iodide dissolved in 30 c.c. of distilled water. If a drop of spermatic fluid or of an aqueous extract of a suspected stain be treated with a drop of this solution and immediately examined under the low-power lens, long rhombic platelets of a dark brown color, fine needles, lance-shaped or ovoid bodies¹ often grouped in rosettes may be observed. A positive reaction is seen many years after the formation of the stain so that a positive result is of value when other sources of cholin are excluded.

Barberio's Test.

Barberio has found that the treatment of a drop of spermatic fluid or an aqueous extract of a suspected stain with a saturated aqueous solution of picric acid gives immediately a precipitate of sharply refractile, yellow, ovoid or needle-shaped crystals which gradually increase in size. This test was supposed to be of much greater diagnostic importance than that of Florence, but the recent work of Fraenkel and Müller has shown that the crystals are not sufficiently characteristic to permit of an absolute diagnosis. They call attention to the fact that substances other than spermatic and prostatic fluids may give similar crystals, but that in such cases these crystals are isolated and form usually on the border of the drop, while with spermatic fluids the crystals are numerous and are formed throughout the specimen. These workers recommend this test for the recognition of prostatic secretions or for the condition of azoöspERMATISM, but caution the worker against making an absolute diagnosis from its presence in medicolegal cases. A negative result does not necessarily exclude the presence of semen.²

It will be seen, therefore, that neither one of the microchemical tests given above should be regarded as absolutely indicative of the presence of semen. It is much better practice to make repeated search for spermatozoa than absolutely to identify a stain as semen by the microchemical method.

Precipitin Test.

Hektoen³ has recently produced a precipitin serum, which appears to be not only specific for the species but, also, semen-specific, that is, limited to constituents of the semen of that species. His method of producing this serum is as follows: Four or five injections of mixed human semen were made intramuscularly in rabbits at intervals of 3 or 4 days, beginning with 2 c.c.

¹ See Lecha-Marzo and Conejero, *Semana Med.*, 1913, XX, 74; also Maestre and Lecha-Marzo, *Ibid.*, 1914, XXI, 776; Vaughan, *Jour. Lab. and Clin. Med.*, 1916, II, 195.

² See Lecha-Marzo, *Arch. internat. de Méd. leg.*, 1913, IV, 341; Harada, *Am. Jour. Med. Sc.*, 1916, CLII, 243.

³ *Jour. Am. Med. Assoc.*, 1922, LXXVIII, 704.

and increasing the quantity by 2 c.c. each succeeding injection. He finds that the best time to bleed the rabbits for serum is from six to eight days after the last injection. As the antigens in this work were not pure semen but mixtures of semen with inflammatory exudates and prostatic secretions, the rabbit antiserum was found, as was expected, to contain precipitins for human serum as well as for human semen. In order to remove the precipitins for human serum proteins from this antiserum, equal parts of the antiserum and dilutions of human serum 1:200 of salt solution are mixed, left at room temperature for about 1 hour and in the icebox overnight, and then centrifuged thoroughly. Hence, two volumes of "treated" antiserum represent one volume of the original antiserum. Likewise, it was found that treatment of the antiserum with gonococcal antigen would remove any precipitin for gonorrheal infection that might have been present from the contamination of the original semen used for immunizing the rabbits. It is to be remembered that the precipitin for human blood will react with seminal fluid. Hektoen finding that precipitates regularly occur in dilutions of 1:8 to 1:45. However, in the "treated" antiserum of Hektoen the precipitins for human serum have been removed.

In this work it has been found that seminal stains on various material are capable of detection by this precipitin test. The clear fluid secured by centrifugation of semen, was examined with positive results in every case in dilutions running from 1:8 to 1:256 or 1:512 of salt solution. In many tests of blood, serum, pus, ascites, fluid, soap, sputum and seminal-prostatic fluids, dried on cotton cloth, the treated antiserum gave positive reactions with the extracts in salt solution only of the spots containing seminal-prostatic fluid. Hence, it seems clear that this test promises to be of practical value in detecting, by its specific precipitin reaction, the presence of human seminal protein in suspected spots and stains.

The technic of this precipitin test is the same as given under the precipitin test for human protein in the Section on Blood.

II. FEMALE SECRETIONS

(1) Vaginal Secretions.

The normal secretion of the vagina is scanty, usually just sufficient to moisten the mucous membrane. It is clear or occasionally opalescent, semi-liquid in character, and is composed largely of mucus and epithelial masses. Its reaction appears variable. As a rule, it should be considered acid in the case of virgins, while in those who have borne children it is usually alkaline.

Little is known regarding the chemical properties of this secretion. From the clinical standpoint the normal vaginal secretion is of importance owing to the fact that it possesses marked bactericidal properties. According to Krönig, pus organisms introduced into the vagina of pregnant women disappear in from four to thirty-six hours. Whether this bactericidal power is due to the reaction of the secretion or to some unknown agent is unsettled. A remarkable fact seems to be that frequent irrigation of the vagina with water or antiseptic solutions decreases the bactericidal power. If this be true, it is questionable whether frequent douching is advisable.¹

¹ See Zweifel, *Monatsschr. f. Geburtsh. u. Gynäk.*, 1914, XXXIX, 459.

Microscopic Examination.

Outside of the great number of large, irregular, stratified, squamous epithelial cells which are constantly found in the vaginal secretion, one observes mucous corpuscles, a few large mononuclear leucocytes, cellular débris, and numerous bacteria. The bacterial flora¹ of the vagina is very extensive. These organisms are normally saprophytic and rarely take on pathologic functions, but they may occasionally do so. Among these bacteria we find the colon bacilli, streptococci, staphylococci, and bacilli which are not unlike true diphtheria bacilli. None of these organisms are particularly important from the clinical standpoint and will be passed with mere mention.² We do find, however, certain organisms which give rise to no particular clinical symptoms, but which are extremely confusing in the examination for the presence of the gonococcus. As will be learned from the later discussion of this organism of Neisser, it appears in the form of biscuit or coffee-berry shaped diplococci, which are both intra- and extracellular and do not stain by Gram's method. In the early cases the intracellular organisms are few in number. Saprophytic cocci often have the same morphology as the gonococcus, but are slightly larger, are variable to Gram's stain, and are rarely intracellular. They are, however, differentiated by the fact that they grow easily upon ordinary media, while the gonococcus requires special media. The more or less normal presence of these saprophytes should be constantly in mind and a diagnosis of gonorrhea made only when clinical symptoms are present to point to the gonococcus. It is wise in all doubtful cases to resort to cultivation, as one may very much regret having made a diagnosis of gonorrhea when such did not really exist. The practitioner should be cautioned to take his smear high up, in the vagina, preferably from the region of the cervix uteri. If this be done as a routine fewer specimens will be found showing these confusing saprophytes. Smears are frequently sent to laboratories for examination which will not show the gonococcus even though present in large numbers in the vagina. It is not sufficient to take a specimen from simple purulent material which may be present in the lower portion of the vagina as the organisms are frequently absent in these locations.³

Pathology.

Blennorrhea.

Physiologically, an increased vaginal secretion (blennorrhea) is seen during sexual excitement, preceding menstruation, and during pregnancy, when a very profuse secretion may be observed. If this secretion contains a large number of epithelial cells and leucocytes, as seen in nonspecific inflammations, it becomes more or less creamy in color and is then called leucorrhea.⁴ This

¹ See Küster, Kolle and Wassermann's Handbuch, 1913, VI, 458.

² McConnell (N. Y. Med. Jour., 1916, CIV, 300) reports the finding of Vincent's fusiform bacillus and spirillum in smears from the cervix. See Noguchi and Kaliski (Jour. Exper. Med., 1918, XXVIII, 559) for a study of the spirochetal flora of the normal female genitalia.

³ See Norris and Mickelberg, Jour. Am. Med. Assoc., 1921, LXXVI, 164; Lehmann, Zentralbl. f. Gynäk., 1921, XLV, 647.

⁴ See Curtis, Surg., Gyn. and Obs., 1914, XVIII, 299; Ibid., 1914, XIX, 25; Du Bois, Rev. Méd. de la Suisse Rom., 1916, XXXVI, 133; Permar, Am. Jour. Obs., 1917, LXXV, 652; Hymanson and Hertz, Ibid., 662; Curtis, Jour. Am. Med. Assoc., 1920, LXXIV, 1706

type of leucorrhea should be sharply differentiated from the true pus secretions observed in the blennorrhagia of gonorrhea, as the former is not necessarily associated with pus formation. In pregnancy a slight catarrhal vaginitis is not infrequent, so that leucorrhea is more apt to appear at such times. If the inflammatory process becomes intense, large shreds of epithelium may be found and ulceration followed by vaginovesical or vaginorectal fistulæ may be observed. Such pathologic findings are usually the result of gonorrhea. In slight catarrhal conditions of the vagina yellowish-gray patches may be seen, which are due to infection with mycotic fungi.

It is not infrequent to find the *trichomonas vaginalis* in the vaginal preparations. This organism has been previously discussed in the section on Feces, to which the reader is referred. The *oxyuris vermicularis* as well as its ova have been reported in the vaginal discharge, but do not seem to have excited any pathologic changes.

Purulent Secretions.

True purulent secretions arising from the vagina are almost always due to the presence of the gonococcus.¹ This organism is accountable for a large number of gynecological conditions, so that it is wise for the practitioner to be able to identify it both from its clinical manifestations as well as by its laboratory detection.

In doubtful cases cultures should be made and a portion of the pus dropped into the eye of a rabbit. The gonococcus itself may later be recovered from the conjunctival exudate.

It is not to be assumed that the finding of the gonococcus in the vaginal discharge is necessarily evidence of a gonorrheal vaginitis or vulvovaginitis. It has been shown that the semen of the male as well as the urethral discharge may contain gonococci and that these may be introduced into a perfectly normal vagina without necessarily setting up gonorrhea. However, this is unusual. The gonococcus may arise from the urethra, the Bartholin glands, acute inflammatory processes of the uterus, or from a ruptured pyosalpinx. In any of these cases the gonococcus may be found in the vaginal discharge, so that the laboratory worker should be guarded in his diagnosis of a vaginitis. Further, suppurative processes which were originally due to the gonococcus may later take on a type of mixed infection or even become of the sterile type. This latter condition is especially observed in old Bartholinitis, metritis, and cystic salpingitis. The organisms usually associated with the gonococcus in the mixed infection are the streptococcus, staphylococcus, colon bacillus and pseudodiphtheria bacillus. In chronic metritis or salpingitis it is not infrequent to find the tubercle bacillus as the causative agent.

Fetid Secretions

In these conditions the pus is usually chocolate colored, has a fatty appearance, is extremely repulsive, is frequently sanguinolent and contains numerous degenerated cells as a result of marked leucolysis. This condition is especially observed in puerperal infection and may be extremely severe.

¹ See Sharp, Jour. Infect. Dis., 1914, XV, 283; Trist and Kolmer, Arch. Pediat., 1915, XXXII, 801.

(2) Uterine Secretions.

Normally, the uterus has no secretion beyond a slight mucoid one which is recognizable clinically. In inflammatory conditions, during normal menstruation, or following abortion or parturition, certain types of discharges are observed which have some clinical importance.¹

Menstruation.

Under normal conditions the menstrual fluid is at first mucoid in character, but within a short time red cells appear and later the discharge takes on almost the character of pure blood. This menstrual fluid should be bright red in color, should contain no clots and should be discharged without causing active pain. This fluid contains red cells, leucocytes, and prismatic epithelial cells showing large areas of fatty degeneration. The duration of the menstrual period is variable, running between two and five days. The amount of blood lost under normal conditions averages about 200 grams, but may be much larger under pathologic conditions. Not infrequently do we find cases in which menstruation is associated with marked pain during more or less of the period of flow. This condition is known as dysmenorrhea and may be associated with the exfoliation of large shreds of mucous membrane, in some cases reported these shreds constituting almost a cast of the uterine cavity. To this latter condition is given the name membranous dysmenorrhea. For the pathologic significance of these abnormal types of menstruation as well as for a discussion of the condition associated with failure of menstruation (amenorrhea) the writer must refer to works on gynecology.

The Lochia.

By this term we have reference to the discharges from the uterine cavity during the puerperium. At first such discharges consist of blood, which may be in the form of clots, and decidual shreds along with epithelial cells which are probably of vaginal origin. This type is known as the *lochia rubra* or *cruentia*. During the next two or three days the discharges become paler and thinner, the red cells diminish and the leucocytes increase, while the decidual shreds may continue approximately the same. This type is known as the *lochia serosa*. After about a week the discharge assumes a grayish or yellowish color and a creamy consistency, the red cells diminishing rapidly and the white cells increasing markedly. Microscopic examination shows, besides the leucocytes and epithelial cells, numerous fat globules and cholesterol crystals. This discharge may continue during the remainder of the period of uterine involution and is known as the *lochia alba*. Under normal conditions the lochial discharge has a faint odor, but is never fetid. If a portion of the placenta or membranes have been retained, the lochia may assume a dirty brownish color and become extremely fetid. After the first two or three days numerous bacteria, such as staphylococci, streptococci and colon bacilli, may be present, but no untoward symptoms exist unless these, along with other saprophytes, give rise to a distinct puerperal infection or sapremia.

¹ Belfield (Jour. Am. Med. Assoc., 1921, LXXVI, 1363) calls attention to the possibility that the endometrium may be infected with syphilis through semen independently of impregnation

Amniotic Fluid.

This is a thin, whitish or pale-yellow fluid containing the constituents of ordinary transudates. The reaction is neutral or faintly alkaline, the specific gravity varies between 1002 and 1008, and the amount of solids rarely reaches 2 per cent. The albuminous bodies are principally vitellin, serum albumin, and traces of mucin, while glucose is absent. Urea and allantoin are present in traces, while creatinin has occasionally been reported.

The amount of amniotic fluid varies between 700 and 1,000 c.c. Under pathologic conditions, however, this amount may be increased or decreased, giving rise on the one hand to polyhydramnios or dropsy of the amnion and on the other to oligohydramnios. For a discussion of these pathologic variations as well as for a treatment of the subject of pathologic changes in the membranes, the writer will refer to works on obstetrics.

Abortion.

The recognition of abortion is usually made by examination of the material discharged from the uterine cavity. Usually one finds blood-clots in which

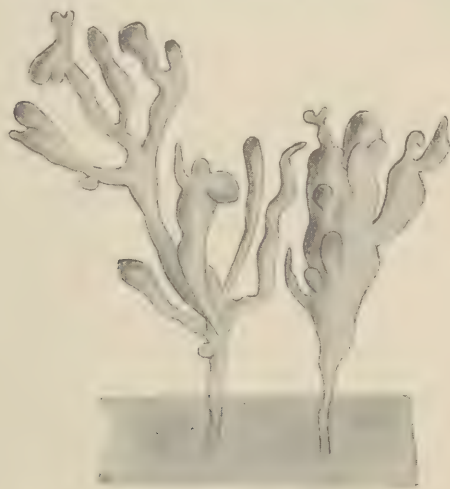


FIG. 116.—Chorionic villi. (McMurrich.)

the villi of the chorion are present. These usually appear as club-shaped masses with epithelial coverings, showing the characteristic capillary network. Moreover, decidual cells are usually present, and may be recognized by their large size, their round, polygonal, or spindle-shaped form, and their irregular and large nuclei with nucleoli.¹

Vesicular Mole.

This condition has been called dropsy of the villi of the chorion, hydatidiform degeneration of the chorionic villi, cystic mole, and myxoma of the placenta. One of its most important symptoms is the expulsion through the vagina of the vesicles forming the degenerated mass. The mole is a mass of pedunculated vesicles which closely resemble a bunch of grapes or gooseberries.

¹ See Lackner, Surg., Gyn. and Obs., 1915, XX, 537.

Each vesicle may vary in size from a millet seed to a large hazelnut and contains a fluid which is usually colorless and limpid, but may be reddish and somewhat dense. Microscopic examination of the tissue shows the peculiar myxomatous degeneration of the chorionic villi.¹

Carcinoma

It is not infrequent to observe, in cases of severe hemorrhage through the vagina, the appearance of occasional shreds, which on microscopic examination show the characteristic appearances of carcinoma of the cervix or body of the uterus. The diagnosis of carcinoma, however, would better be made upon sections removed by the surgeon rather than upon shreds found in the hemorrhagic fluids. Although a diagnosis may be at times possible, it should be somewhat guarded unless the clinical symptoms are distinctive.

¹ See Westermarck, *Hygiea*, 1914, LXXVI, 801; Iraci, *Policlinico*, 1915, XXII, 1505; Amann, *Monatsschr. f. Geburtsh. u. Gyn.*, 1916, XLIII, 11; Bar, *Arch. Mens. d' Obs. et de Gyn.*, 1916, XXI, 49; Caturani, *Amer. Jour. Obs.*, 1917, LXXV, 591; Lopez, *Med. Ibero*, 1919, IX, 5; Kirtley, *Northwest Med.*, 1921, XXI, 31; Swee, *China Med. Jour.*, 1921, XXXV, 264; Henderson and Sherrill, *Kentucky Med. Jour.*, 1921, XIX, 311; Hannah, *Southern Med. Jour.*, 1921, XIV, 572; Hinselmann, *Monatsschr. f. Geb. u. Gynäk.*, 1921, LV, 1; Velasco, *Philippine Islands Med. Assoc. Jour.*, 1921, I, 153; Frey, *Schweiz. med. Wehnschr.*, 1922, LII, 201; Novak, *Jour. Am. Med. Assoc.*, 1922, LXXVIII, 1771.

CHAPTER VIII

THE BLOOD

I. GENERAL CONSIDERATIONS

The blood is perhaps the most important tissue in the body, inasmuch as it is at once the purifier and the nutritive source of the cell. Any normal or abnormal product of cellular activity finds its way ultimately into the blood, either to be taken up by the assimilatory organs or to be thrown out by the excretory ones. While but relatively few disease processes are associated with diagnostic findings in this tissue, yet many are characterized by definite manifestations which are invaluable aids to the clinician.

It is, therefore, of the utmost importance that we should have a proper knowledge of the normal blood in order better to understand the various phases which characterize abnormal blood and which give to hematology such an interesting and important position in diagnosis.¹ While it is true that some of the methods involved in hematological examinations require definite apparatus and a large experience for their proper interpretation, yet the results obtainable from the ordinary routine blood examinations are so invaluable, being in some cases pathognomonic, that no practitioner should consider himself fitted to give his patients the proper service without being equipped with a clear working knowledge of the methods of examination and the findings of normal and of abnormal blood.

It is essential to remember that certain physiologic as well as pathologic conditions influence the quantity and quality of the blood. So great are the effects of digestion, exercise, nervous factors, massage, cold, heat, sweats, dysentery, constitutional and specific diseases, that one does not wonder at the many conflicting reports of cases showing widely varying hematological findings. As Grawitz has pointed out, no conclusion should be drawn from an examination of the blood without taking into consideration the physiologic and pathologic condition of the patient.

It is a pleasure to observe in these days the tendency toward a more rational and thorough study of the plasma, the so-called "intracellular fluid" of the blood. We have forgotten, in our enthusiasm over the many valuable findings obtained from histological investigations, that the relations of the fluid portions of the blood are, in some cases, of quite as much importance as are the variations in the cellular elements. It is necessary only to cite the work on blood chemistry, lysins, precipitins, agglutinins, opsonins, etc. to show the value of a more extended study of the plasma or serum.

Regarding the technic of blood examinations, the writer will have much to say later, but he wishes to impress upon his readers one point which has

¹ See Lucas (*Jour. Am. Med. Assoc.*, 1921, LXXVII, 332) for a discussion of the physiology of the blood in infancy and childhood.

been well expressed by Türk, namely, an indispensable basis for the proper utilization of any diagnostic, prognostic, or therapeutic method of clinical examination is a knowledge of the absolute limitations of the method. Reliable results can, however, be obtained only by those who are thoroughly familiar with the principles as well as with the technic and the little "knacks" of the method used. It will be found, when the attempts are made to apply the methods outlined, that quite as much depends on the exactitude with which the separate details are carried out as upon the selection of the method itself. It is not to be expected that a first trial will yield exact results or that a few determinations will perfect one in the methods of examination. Experience is the only teacher that can equip one with the skill and power of interpretation necessary to cope with the many difficulties to be overcome in the hematological investigations.

II. PHYSIOLOGY AND CHEMISTRY

(1) Blood Formation and Blood-forming Organs.

While it is impossible in a general work of this character to go into great detail regarding the formation of the blood, yet it seems to the writer that a brief discussion of this subject is extremely valuable both to the student and practitioner.¹

Red Corpuscles.

According to Kölliker, the first blood-corpuscles have their origin, in embryonal life, in the embryonic heart and blood-vessels. They appear as nucleated colorless cells, which later develop into colored corpuscles by the appearance of hemoglobin in some of the cells of the mesodermal cord, which cells go to form the first capillaries. Upon the formation of these vessels the cells lie within them as nucleated reds. At this time there are no true leucocytes and none appear until after the complete formation of the red cells, which is advanced as an argument against Pappenheim's theory of single origin of red and white cells. It will thus be noted that the vessel wall and the primitive erythrocyte have a common origin in the mesodermal cord, the peripheral cells going to form the endothelium of the vessel and the internal cells the corpuscles. Up to the end of the fourth or fifth week of embryonal life all of the red cells are nucleated; while from that time on the relation of the nonnucleated to the nucleated forms gradually increases until at birth few if any nucleated cells obtain.

In later embryonal life (about the third month), the liver becomes the chief seat of blood formation. During the fifth month, the spleen² and lymph-glands take up this work, and finally the bone-marrow becomes the seat of such activity.

In extrauterine life, the bone-marrow is the chief point of formation of the red cells, but under pathological conditions the spleen and liver may assume their embryonic functions. It appears that the formation of nucleated

¹ See Gruner, *The Biology of the Blood Cells*, New York, 1914.

² See Morris, *Jour. Exper. Med.*, 1914, XX, 379; Hirschfeld and Weinert, *Berl. klin. Wchnschr.*, 1914, LI, 1026.

reds in the adult is practically the same as in the embryo and that, at all periods of life, the red cell is the product of several series of mitoses of a colorless mesoblastic cell. The difficulty of tracing this series, from the large nucleated red cell to the colorless mesoblastic "mother-cell" in the marrow, has given rise to the diverse opinions now held regarding the ultimate development of the red corpuscles.

Leucocytes.

The earliest indications of the formation of leucocytes are seen in the presence of primary wandering cells, of mesodermal origin, which are found principally in the loose connective tissues of the early embryo. Though of mesodermal origin they are, from the first, quite distinct in morphology and, apparently, in function from the capillary endothelium and fixed connective tissue cells. Their development has been traced by Ziegler to masses of mesodermal cells surrounding the cords from which the capillaries are formed. It thus seems that originally the parent leucocytes lie outside the vessels, into which they make their way by virtue of ameboid powers.

Most observers find that the primary wandering cells produce, by mitotic division, one or more generations of colorless cells which gradually approach, in morphology, the early basophilic leucocytes of the circulation. Denys, Löwit, Ziegler, von der Stricht, and others claim that red cells and white cells develop from separate series of cells, which have become differentiated from the primary mesodermal cells with the first appearance of blood and blood-vessels. Kostiannecki, Muller, Schmidt, Saxer, Pappenheim, and others believe that the primary wandering cell persists in the blood-forming organs as the parent of both red and white cells.

Before the leucocytes begin to appear in the circulation, mitotic figures are abundantly seen in the primary wandering cells in various situations. These are gathered in groups, first in the loose connective tissues of various regions, where lymph nodes subsequently develop; but the chief seat of the production of the leucocytes is found in the embryonal liver. In both situations the wandering cells are found in lymph and blood capillaries, in the interstices of the connective tissues, and between the liver cells. In later embryonal life the process is gradually transferred from the liver to the lymphoid and adenoid tissues, as indicated by the development of lymph nodes, spleen, marrow, and thymus. Under normal conditions, the reproduction of leucocytes, in the adult, is limited to the lymphoid structures both of the lymph-glands and bone-marrow.

(2) Total Volume of Blood.

The various methods which have been advanced for the estimation of the total quantity of blood in the body are subject to such wide variations that they have yielded little exact information regarding this subject. The procedures advocated by Valentine, Vierordt, Buntzen, and Thibault have an error sufficiently great to exceed the physiological and pathological variations of the blood. By these methods, the quantity of blood in the body has been estimated as equal to one-thirteenth of the body-weight. The so-called clinical methods of Quincke or of Tarchanoff are of purely theoretical

interest, because certain factors, such as the appearance of the patient or the volume of the pulse, are taken into consideration in making a rather unreliable guess as to the total quantity of blood in the body (Buckmaster).

By the use of a reliable method, introduced by Haldane and Smith,¹ the total volume of blood may be fairly accurately estimated. This method is based on the following points. The capacity of hemoglobin for oxygen and for carbon monoxid is identical. On the assumption that none of this latter gas is oxidized in the body, and that no substance in the blood, other than hemoglobin, unites with it, the experimenter is able to determine the CO capacity and hence the O capacity of the blood. This method has, however, little clinical application and will be left with reference to the original work. In the cases studied by Haldane and Smith by this method, the average value was 3,240 c.c. or, on the basis of a specific gravity of 1,060, about 3,434 grams. This yields, according to Smith, a figure ranging between one-sixteenth and one-thirtieth of the body-weight.² In obese persons the volume of blood is less, proportionately, than in the more normal specimens of mankind.

The question of the volume of blood in the body is of great importance in the study of the changes taking place in this tissue. It must be remembered that the number of red or of white cells in a cmm. of blood will depend upon the total amount of blood present. If for any reason the volume is diminished or increased, corresponding changes, in the inverse sense, will be observed in the number of the cellular elements per cmm. It seems to the writer, therefore, that certain factors, not ordinarily taken into account in blood examinations, should be known before any definite report is made upon a blood count. It may be readily seen that a concentration of the blood, due to hemorrhage, diarrhea, sweating, etc., will lead to an apparent increase in the number of corpuscles.

Certain physiologic and pathologic conditions lead to definite changes in the volume of the blood, as such, or of some of its constituents. As Plehn has recently shown, the volume remains quite constant or is adjusted through the activity of the capillary endothelium and through the influence of the nervous system. However, definite changes of a more or less transitory nature do occur and exert marked influences on the results of blood examinations as well as upon many pathologic conditions.³

Oligemia.

By this term is meant a reduction in the total volume of blood, both as

¹ Jour. Physiol., 1896, XX, 497; Ibid., 1900, XXV, 333. See Salvesen (Jour. Biol. Chem., 1919, XL, 109) for a new method of determining blood volume. His determinations show the blood volume to average 1/16.8 of the body weight.

² Keith, Rowntree and Geraghty (Arch. Int. Med., 1915, XVI, 547) consider that the blood constitutes 8.8 per cent. (1/11.4) of the body-weight. Normal individuals have approximately 85 c.c. of blood per kilogram. In this connection see Hooper, Smith, Belt, and Whipple, Am. Jour. Physiol., 1920, LI, 205; Smith, Ibid., 221; Dawson, Evans, and Whipple, Ibid., 232; McQuarrie and Davis, Ibid., 257; Löwy, Zentralbl. f. inn. Med., 1920, XLI, 337; Boenheim and Fischer, Ibid., 553; Smith and Mendel, Am. Jour. Physiol., 1920, LIII, 323; Denny, Arch. Int. Med., 1921, XXVII, 38; Bock, Ibid., 83; Erlanger, Physiol. Reviews, 1921, I, 177; Arnold, Carrier, Smith and Whipple, Am. Jour. Physiol., 1921, LVI, 313; Lee and Whipple, Ibid., 328; Smith, Arnold and Whipple, Ibid., 336; Franke and Benedict, Jour. Lab. & Clin. Med., 1921, VI, 618.

³ See Kämmerer and Waldmann, Deutsch. Arch. f. klin. Med., 1913, CIX, 524; Miller, Keith and Rowntree, Jour. Am. Med. Assn., 1915, LXV, 770; Uthman, Am. Jour. Dis. Child., 1920, XX, 366; Marriott, Ibid., 461; Lucas and Dearing, Ibid., 1921, XXI, 96.

regards the liquid and the cellular portions. This condition is most frequently noticed after profuse hemorrhage¹ and may be so marked that death results. In other cases, in which the hemorrhage is less extensive, the loss of blood is made up by osmosis from the lymph spaces into the capillaries and, later, by an increase of the cellular elements due to compensatory activity of the hematopoietic organs.

Plethora.

The opposite of the preceding condition is known as plethora, a state characterized by an increase in the total volume of blood. There has been much discussion as to whether a true plethora exists, but there can be little doubt that a transitory *plethora vera* may occur as a result of direct transfusion of blood, and also, according to Bergmann and Heissler, who have established the fact that there is a direct ratio between the volume of blood and the size of the heart, on the one hand, and the muscular development of the subject, on the other, as the result of increased muscular activity, provided the loss of fluid by perspiration is not excessive. Such a plethora disappears, of course, in a very short time.

In this discussion we must distinguish between a serous and a cellular plethora. By the former is meant an increase in the volume of blood due to excessive quantities of its liquid and soluble constituents; while by the latter we understand an increase in the number of corpuscular elements, that is a polycythemia. Serous plethora is most frequently observed in organic lesions of the kidneys and of the heart, in which a diminished elimination of water and inorganic constituents is noted. This condition is usually of a transient duration, as the volume of blood is soon restored to normal by intracapillary transudation and by diffusion. Osmotic effects must also be taken into consideration here, inasmuch as the salts will tend to diffuse out from the blood and will consequently draw water after them, giving rise, under certain conditions, to dropsical effusions of a more or less transient duration.²

Hydremia.

Another condition of the blood is frequently observed, in which an increase in the quantity of the liquid constituents is observed. This is known as hydremia and is different from serous plethora, as the latter carries with it an increase in the saline as well as in the watery portion of the blood. In hydremia the specific gravity of the blood is reduced, while in serous plethora it is increased. As Engel³ has shown, the estimation of the coefficient of refraction of the blood serum is a reliable method for the clinical study of the subject of the water content of the blood.

Hydremia may be produced by any factor which changes the normal relationship of the blood constituents in such a way that the watery portion

¹ See Hogan, Jour. A. M. A., 1915, LXIV, 721; Bogert, Underhill and Mendel, Am. Jour. Physiol., 1916, XLI, 219; Scott, Jour. Physiol., 1916, L, 157; Guthrie, Jour. A. M. A., 1917, LXIX, 1394; Arch. Int. Med., 1918, XXII, 1; Penn. Med. Jour., 1918, XXII, 123; Meek and Gasser, Am. Jour. Physiol., 1918, XLVII, 302; Robertson and Bock, Jour. Exper. Med., 1919, XXIX, 139; Aebly, Cor. Bl. f. Schweiz. Aerzte, 1919, XLIX, 1393; Lee, Am. Jour. Med. Sc., 1919, CLVIII, 570; Gasser, Erlanger and Meek, Am. Jour. Physiol., 1919, L, 31 and 140.

² See Krumbhaar and Chanutin, Jour. Exp. Med., 1922, XXXV, 847.

³ Magyar Orvosi Arch., 1906, VII, 555.

is relatively increased. It is in these more or less physiologic states that we are apt to observe the greatest variation in the blood counts. As the cellular elements are not simultaneously increased, the drop of blood under examination contains relatively fewer cells than normally. The most common physiologic causes of hydremia are the ingestion of large quantities of fluids, saline transfusions, and vasomotor dilatations as a result of exercise or nervous influences. In severe anemias we find the watery portion of the blood relatively increased. In dropsical states, following cardiac or renal lesions, we often observe such a condition, whose duration will depend, of course, upon the etiologic factors of the trouble.

Anhydrema.

This is a condition characterized by a diminution in the liquid constituents of the blood. There is no change in the cellular elements and hence a blood count will show an erroneous increase in the number of cells. In this condition the specific gravity of the blood is naturally increased.

Anhydrema follows any condition which results in the excessive loss of fluid from the body, as, for instance, that following profuse diarrhea, polyuria, sweating, vomiting, and effusions into the various serous cavities of the body. According to Oliver, this state may be caused by influences which increase the arterial tension and hence bring about an increase in the passage of water from the vessels into the tissues. Thus, for instance, we may observe anhydrema following local and general exercise, massage, bathing, etc.

(3) Volume Relations of Cells of Plasma.

The study of the relationship between the cellular and the intracellular portions of the blood is a comparatively recent addition to the technic of blood examinations. This determination is based on the principle that the corpuscles may be thrown by centrifugal force to the distal end of a calibrated tube, while the plasma will collect in the proximal portion. If the tube be properly calibrated, the percentage relations of the cells and plasma may be readily ascertained. These ideas were used by Hedin in elaborating the earlier methods of Blix.

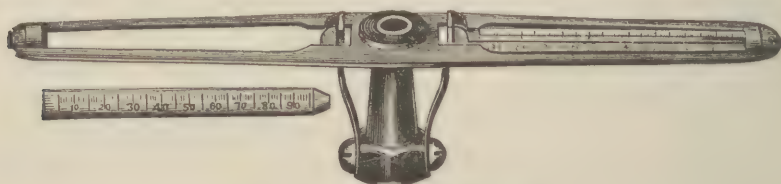


FIG. 117.—Daland's hematocrit.

Daland's Hematocrit.

Daland has introduced a modification of the clumsy model of Hedin and has succeeded in simplifying the technic to such an extent that this method is directly applicable to clinical use. His instrument is shown in the accompanying cut.

One of the calibrated glass tubes is fitted with the rubber tubing and is filled with blood from the ear or finger. The forefinger, smeared with a

little vaselin, is then placed over the beveled end of the tube and the rubber tubing withdrawn. Insert the tube into one arm of the frame, the other arm of which should carry the second tube filled in the same manner, in order to balance the instrument and to control the reading. Rotate the spindle for three minutes at such a rate of speed as will insure 10,000 revolutions per minute (80 revolutions of handle). In this way the corpuscles are separated from the plasma and are distinguishable as a distinct column, which may be read off directly from the graduations of the tube. These divisions will give the percentage relations of the cells and plasma as the tube is divided into 100 equal portions, each division of the scale representing approximately 100,000 cells. This latter makes it possible to make a rather rough blood count with this instrument, but it is to be remembered that accurate results cannot follow, as we find such variations both in the size and elasticity of the cells in the different conditions in which the number of cells is most sought.

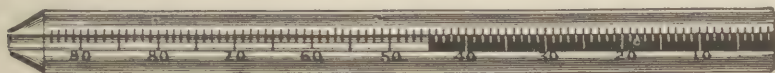


FIG. 118.—Hematocrit tube.

Volume Index.

Capps¹ has introduced the conception of volume index, that is the quotient of the volume per cent. as obtained with the hematocrit, and the blood count in terms of per cent. Sahli advises the use of the expression volume quotient or volume value for this factor.

By means of the hematocrit the volume of the red cells, as compared with that of the whole blood, is taken. In normal cases this is about 50 per cent. which is reckoned as one. Hence the volume of the red cells may be obtained directly in percentage value. The red cells are then counted by the method to be later outlined, and the result is expressed in percentage by comparing this count with a so-called normal one of 5,000,000 red cells. By dividing the volume of red cells (in per cent.) by the per cent. of red cells, Capps obtains his volume index of the red cells.

In normal conditions this quotient is one. According to Capps, an increase of this index is a constant factor in pernicious anemia. The color index never exceeds the volume index in such cases, which fact shows that there is no supersaturation of the corpuscles with hemoglobin. In primary and also in secondary anemia this factor is diminished. Here we find the color index often falling below the volume index.

This method may be used in detecting various pathological conditions of the blood. According to Emerson, it is used in the Johns Hopkins Hospital in ascertaining the presence of lipemia, cholemia, or hemoglobinemia. It would seem to the writer that it could be employed with advantage as a routine procedure, especially in hospital practice. The osmotic pressure of the plasma plays a great rôle in this determination, as the concentration of the blood may be such as to cause swelling or shrinkage of the cells. As

¹ Jour. Med. Research, 1903, X, 367. See, also, Ege, Biochem. Ztschr., 1920, CIX, 241; Ibid., 1921 CXV, 109; Norgaard and Gram, Jour. Biol. Chem., 1921, XLIX, 263.

Capps has found, normal red cells with a volume index of one have their discoplasm saturated with hemoglobin. Hence, if the hemoglobin index becomes greater than one, an enlargement of the red cells is indicated. Upon the other hand, the color index may fall, regardless of a corresponding lowering of the volume index. It follows, therefore, that, if the color index is above normal, the volume index must also be increased; while, if the hemoglobin index is below normal, the volume index is not necessarily diminished (Sahli).

(4) **Methods of Obtaining Blood.**

The method of obtaining blood for examination depends upon the amount desired and on the examination which is to be made. For ordinary routine work only a few drops are necessary, while for chemical, serological or bacteriological investigations 2 to 20 c.c. may be required.

In obtaining the blood no set rule can be laid down as to the proper place from which to take the specimen. We should select the part which promises the best results, avoiding naturally the points which are cyanosed, eczematous, edematous, hyperemic, cold, unduly heated, or, in other words, any part which is not normal. The ear usually furnishes the best results, in the writer's opinion, because its puncture is relatively painless, it is easily accessible (which point is often of importance in attempting to obtain blood from those who are comatose or who refuse to submit their hand for puncture), and because the patient, who may be easily affected by the sight of blood, can not see the drop. If the lobe of the ear is thick it is usually pricked on the flat side, but if it be thin it is well to make the puncture on the edge and parallel to the surface. Some workers prefer the palmar surface of the ball of the middle or second finger of the left hand and others advise pricking the arm over a small superficial vein. In cases in which repeated examinations of the blood are to be made the parts should be varied in order to prevent soreness and also to avoid anesthetics which occasionally follow repeated use of the same site of puncture.

Quite a number of special forms of blood needles, as, for instance, those of Francke and of Daland, are on the market, and each of them has its advocates. Many of them are in the form of special holders, which permit a puncture of a desired depth to be made without any danger of going too deep. These are to be recommended to those only who seem unable to control their stab. The writer does not find that the results obtained by the student are any better with such instruments than are those following the use of the ordinary Hagedorn surgical needle. In lieu of any better article, a clean steel pen with one nib broken will yield admirable results. The one thing to bear in mind in selecting a needle for blood work is that the point have a cutting-edge and should not be round or sharp nor should it be too long or slender.

Having decided upon the part from which the blood is to be taken, this surface is washed carefully with water and alcohol or ether and is then thoroughly dried. Avoid any undue rubbing as this will cause hyperemia and will introduce an error into the work. It is, as a rule, unnecessary to sterilize

the needle but if it seems advisable to do so on account of the patient's attitude, the sterilization is best done by heat, hydrogen peroxid or alcohol, allowing the needle to cool before making the puncture. In this latter part of the technic, much depends on the amount of blood desired as regards the use of a short quick stab or a slow steady puncture. The latter procedure usually yields more blood but is more painful. It is much better to prick the patient too deeply, going even through the lobe of the ear, than it is to subject him to repeated punctures. The part pricked should not be squeezed nor held in a position which will cause an abnormal circulation. If the puncture is successful, the blood will come out in good-sized drops. The first of these are wiped away and subsequent ones used for the examination. As often happens, the blood coagulates fairly quickly, so that the coagulum should be wiped off with a little alcohol followed by a dry cloth. It is important to remember that a patient with hemophilic tendencies or history may bleed very easily from a very slight puncture. Care should, therefore, be taken to question the patient regarding the ease with which blood flows from a wound and also regarding the history of "bleeders" in the family.

If considerable blood is desired, resort must be made to venous puncture. The site of this operation is usually the median basilic vein at the bend of the elbow. This vein may be made more prominent by tying a tight bandage around the arm, but the bandage should be removed before the blood is withdrawn, except when serum reactions are to be studied. The site of puncture must be thoroughly cleansed, using the precautions observed before any surgical operation. Likewise, the needle and the aspirating instrument must be absolutely sterile before puncture is made. The instrument best adapted for this purpose is, in the writer's experience, the Lür syringe, which is made of glass with a tightly fitting glass plunger and adjustable platinum needle. From 2 to 20 c.c. of blood are withdrawn and immediately placed in work. The question of the bacteriological examination and of serum reactions will be discussed in a later section.

(5) Physical Properties.

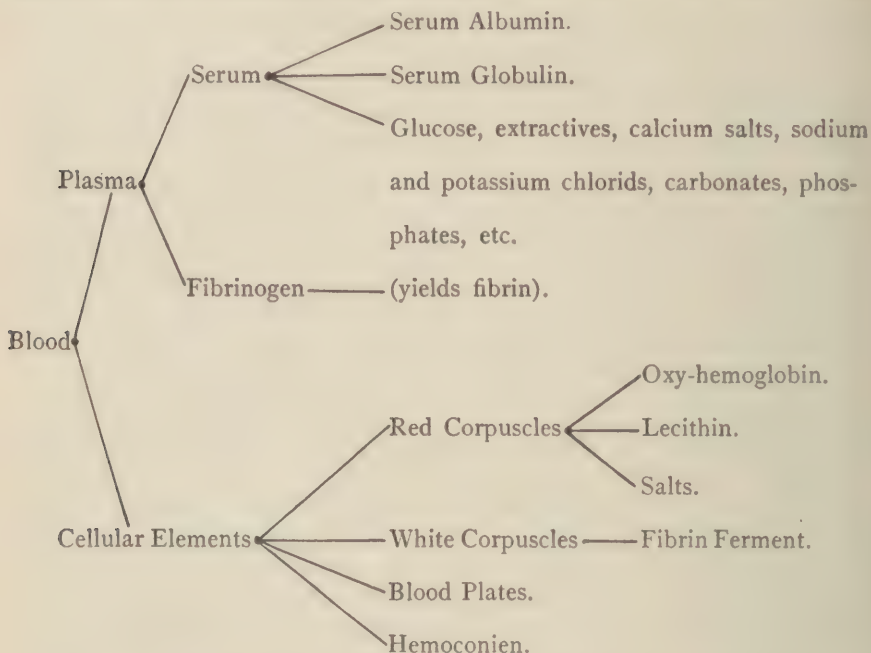
The blood must be regarded as a fluid tissue, consisting of a transparent liquid, the plasma or *liquor sanguinis*, in which are suspended the corpuscular elements, erythrocytes, and leucocytes. Besides these latter cells we find two constituents, the blood plates of Bizzozero and the hemoconien (blood dust), which are hardly to be considered as true corpuscular entities.

As it flows from the vessels, blood is a thick, viscid, red liquid, having a peculiar odor, a salty taste, and an alkaline reaction to litmus. If allowed to stand it shows, unless certain precautions are observed, the peculiar phenomenon of coagulation. In this process the blood is separated into two portions, the cellular elements and the plasma, the latter changing, as the



FIG. 110.—
Blood needle.

process goes on, into serum and the clot (fibrin), which holds the corpuscles in its meshes. In the diagram given below, adapted by Webster and Koch, may be seen the composition of the blood.



(4) Color.

The color of the blood is due to the presence in the erythrocytes of an iron-containing albuminous substance, hemoglobin, which has remarkable affinity for oxygen and other gases. This latter property enables this pigment to play one of the most important rôles in the body economy. Arterial blood is bright red in color, while venous blood shows a purplish-blue tint. These variations are due entirely to the relative proportions of oxygen and of carbon dioxid in the two types of blood. Many different shadings are observed, physiologically and pathologically, in the coloration of the blood, and each is due to some combination of hemoglobin with normal or abnormal substances.

The presence of large numbers of red corpuscles in the blood gives rise to a characteristic opacity of this tissue. If, for any reason, such as admixture of blood with water, dilute salt solutions, urea, ether, snake venom, extract of mushrooms, etc., the blood loses its opacity, the change is due to the dissolving out of the hemoglobin from the red cells. This is the well-known phenomenon of "laking" or, better, of hemolysis, about which we will have something to say later.¹

The normal color of the blood is often changed in pathological conditions. Thus, in anemia the blood is pale and watery; in leukemia it may be milky; in diabetes buff-colored; while in poisoning with potassium chlorate it is chocolate-colored and in that with carbon monoxid it is bright red.²

¹ See Guthrie, *Jour. Lab. and Clin. Med.*, 1917, III, 87.

² See Gaisböck, *Med., Klinik*, 1912, VIII, 1906.

(B) Odor.

The odor of the blood is peculiar and indescribable. This *halitus sanguinis* is due to the presence of certain volatile fatty acids and may be rendered more distinct by the addition of concentrated sulphuric acid, which increases the volatility of these acids (Barruel's test).

(C) Reaction.

If we are to accept the teachings of physical chemistry, that the alkalinity of a solution is due to the presence of free hydroxyl (OH) ions and that its acidity depends on the surplus of free hydrogen (H) ions, we must grant that the blood is a practically neutral fluid. If, however, we have in mind the acid-combining power of the blood, we must regard the reaction of this tissue as alkaline. It is certain that the blood shows both acid and alkali combining powers due to the presence of protein constituents as well as to both acid- and alkali-reacting substances, the measure of such powers being dependent on the indicator used in the estimation. As the combining power for acids is greater, in the case of blood, than it is for alkalies, the reaction, as judged by titrimetric methods, must be alkaline.

The normal free or diffusible alkalinity of the blood is due to the presence of disodium hydrogen phosphate (Na_2HPO_4), sodium bicarbonate (NaHCO_3), and sodium carbonate (Na_2CO_3). This total diffusible alkali constitutes, according to Brandenburg, about 20 per cent. of the entire alkalinity and may be measured by dialyzing against known alkaline solutions and observing the concentration at which the strength of the known solution does not change. This factor represents the so-called alkaline tension of the blood and remains fairly constant, in normal cases, at about 60 mg. of NaOH per 100 c.c. of blood, while in pathologic conditions, such as uremia, diabetes, etc., it is somewhat reduced.

Besides this diffusible alkali, the blood contains nondiffusible alkali bound to the proteins. This portion represents normally about 80 per cent. of the total alkalinity, and is dependent largely on the cellular content of the blood, as the soluble protein constituents are not generally subject to wide variations. The fluctuations in this nondiffusible alkali are no doubt accountable for the great differences in the figures given for the alkalinity of the blood.

The subject of the reaction of the blood is one which should furnish, if properly studied by reliable methods, much valuable data upon subjects which are now very obscure. As Moore and Wilson¹ have pointed out, the titration methods do not give us the true neutrality of the blood, but rather the amount of alkali or of acid which may be added to it without raising the hydroxyl or hydrogen concentration above certain low limiting values. The reaction of the blood depends on the indicator used for the determination of the neutral point and cannot be definitely measured by any method which employs an indicator for such purposes. These writers have introduced the term "reactivity" to indicate the property, possessed by the

¹ Biochem. Jour., 1906, I, 297. See, however, Sellards, Bull. Johns Hopkins Hosp., 1914, XXV, 101. Rieger (Jour. Lab. & Clin. Med., 1920, V, 668) has devised a method for the determination of the acid-fixing power (oxydesis) of the blood, by estimating the greatest amount of acid that can be added to a unit volume of blood without damage to its corpuscles.

blood, of combining with both alkalies and acids in such a way as not to raise its ionic composition.

In discussing the methods, which are now in use, for the determination of the reaction of the blood, one must have in mind just what is meant by the terms "acid" and "bases," from the physico-chemical point of view. When the molecule of hydrochloric acid, for instance, is dissolved in water it splits or dissociates into two portions called ions, H and Cl, each bearing an electric charge of opposite character. The electric charge of H, as well as of all metals, is positive, while that of Cl, as of all acid groups, is negative. As unlike charges of electricity are attracted by each other, it follows that the H ions will collect at the negative pole, or cathode, while the Cl ions will pass to the positive pole, or anode. It is for this reason that positive ions are called "cations" and negative ions "anions." The more modern definition of an acid is a solution which contains an excess of hydrogen over hydroxyl ions; neutrality is indicated by an equal number of hydrogen and hydroxyl ions; while basicity (alkalinity) is denoted by an excess of hydroxyl ions over hydrogen ions. In the combination of acids with bases the hydrogen ions unite with the hydroxyl ions forming water, and thus removes the free ions from the field. It is evident, therefore, that the most strictly neutral solution must be pure water, in which the H and OH ions are exactly balanced. It has been shown that, even in the purest water, a slight degree of dissociation occurs, giving us free H and OH ions. The equation, used in physical chemistry, to indicate the dissociation of water is the following, which is reversible; $\text{H}_2\text{O} \rightleftharpoons \text{H} + \text{OH}$. From the above considerations, it is evident that there must be an equilibrium between the H and OH ions, which is expressed in the equation, $(\text{H}) \times (\text{OH}) = K_w$, that is the product of the free H ions by the free OH ions is a constant, which is called the "dissociation constant of water." When the concentration of these ions is multiplied, in the purest water, the product is 1.2×10^{-14} , which means that there are 1.2 gram molecules of hydrogen and hydroxyl present in 10,000,000,000,000 liters. As H and OH are to be considered equal, the H ions must be 1.2×10^{-7} , which signifies that this is present in sufficient quantity to form a 0.000,000,12 N solution, or in other words, 1.2 grams of H in 10,000,000 liters. In the study of solutions, the standard of concentration is taken as the "Normal" solution, which, by definition, contains the equivalent of 1 gram of hydrogen in 1 liter of the solution.

If the dissociation constant of water, as mentioned above, is to remain a constant, there must be present in every aqueous solution some H ions and there must be maintained a balance between the concentrations of H and OH. For this reason, when acid is added to water the concentration of H ions necessarily rises, but the concentration of OH ions falls correspondingly, so that, as in pure water, $\text{H} \times \text{OH} = K_w = 1.2 \times 10^{-14}$. No matter how acid or alkaline a solution may be, the products of these concentrations must be the same. It follows, therefore, that solutions with H ion concentrations greater or OH ion concentrations less than 1.2×10^{-7} are called acid solutions; while those with H ion concentrations less than or OH ion concentrations

greater than the above are called alkaline solutions. It will thus be seen that acidity and alkalinity may both be expressed in terms of H ion concentration. In order to avoid writing large figures for the acidity or alkalinity in expressing the normality of a solution, the logarithmic notation is used, the H ion concentration (C_H) being expressed in negative coefficients of 10. Thus a 1/10,000,000 N solution would be expressed as 10^{-7} . It has, however, become customary to use the notation suggested by Sorensen, for a matter of convenience, and designate the H ion concentration by P_H , which is the logarithm of the reciprocal of the H ion concentration. Thus the H ion concentration of a 1/10,000,000 N solution would be written, P_H^7 . As both notations are used in the literature, it is necessary to know how to convert them for purposes of comparison. A convenient method is to find the logarithm of the number of gram molecules of H and subtract this from the characteristic. Thus to convert the expression 1.2×10^{-7} into terms of P_H , the logarithm of 1.2 is 0.060206; hence $7 - 0.0602 = 6.94$. The following table will serve to indicate these relations:

P_H	C_H
5.0	1.0×10^{-5}
5.1	7.9×10^{-6}
5.2	6.3×10^{-6}
5.3	5.0×10^{-6}
5.4	4.0×10^{-6}
5.5	3.2×10^{-6}
5.6	2.5×10^{-6}
5.7	2.0×10^{-6}
5.8	1.6×10^{-6}
5.9	1.2×10^{-6}
6.0	1.0×10^{-6}
6.1	7.9×10^{-7}
6.2	6.3×10^{-7}

As P_H increases, C_H decreases, and vice versa. Hence, values lower than 7 denote increase of acidity and values greater than 7 denote lessened acidity or alkalinity. In the case of solutions of varying normality, this may be seen from the following table

P_H^1 indicates N/10 acid	
P_H^2 indicates N/100 acid	
P_H^3 indicates N/1000 acid	
P_H^4 indicates N/10000 acid	
P_H^5 indicates N/100000 acid	
P_H^6 indicates N/1000000 acid	
P_H^7 indicates N/10000000 acid	(Neutrality)
P_H^8 indicates N/10000000 alkali	
P_H^9 indicates N/1000000 alkali	
P_H^{10} indicates N/100000 alkali	
P_H^{11} indicates N/10000 alkali	
P_H^{12} indicates N/1000 alkali	
P_H^{13} indicates N/100 alkali	
P_H^{14} indicates N/10 alkali	

On the addition of an acid or an alkali to water or normal salt solution a change in P_H must occur. However, when acid or alkali is added to whole blood, plasma, or serum, the H ion concentration does not change to any appreciable extent until quite an excess has been added. It is for this reason that the ordinary titrimetric methods for determining the alkalinity of blood are of little avail. This is simply another way of expressing the fact

that in health and in disease the reaction of the blood does not vary much from the usual value of P_H ⁷, the factor being a physiologic constant. Even the slightest increase in C_H is to be regarded as incompatible with life if continued for any length of time. The power of certain solutions, such as blood, to resist change in reaction, on addition of acids or alkali, is due to what is styled the "buffer" or "tampon" action of these solutions. In the case of whole blood, the presence of both alkaline and acid phosphates, of carbonates and bicarbonates, and of proteins, which serve as both weak acids or bases, affords the maximum opportunity for the display of this buffer action. In so far as the plasma is concerned, this buffer action is principally due to the presence of bicarbonates. Through the work of several investigators, especially Henderson and his co-workers and Van Slyke and his colleagues, the influence of these substances has been very carefully studied and methods evolved for the determination of the bicarbonates of the blood and their importance as indicators of varying degrees of acidosis. It has been found that the hydrogen ion concentration of the blood is directly proportional to the ratio existing between CO_2 in solution as H_2CO_3 and sodium bicarbonate ($NaHCO_3$) multiplied by a constant. This may be expressed as

$$P_H = \text{the molecular ratio } \frac{H_2CO_3}{NaHCO_3} \cdot \text{This ratio is normally } \frac{1}{20}.$$

It is evident, therefore, that while P_H is maintained with great tenacity by the normal organism, that the bicarbonate value decreases progressively as soon as the normal excess of bases over acids begins to be noted. As will be seen later the organism, through the respiratory mechanism, rids the body of the excess of CO_2 formed by the action of acids upon the bicarbonates and thus maintains the normal ratio between the H_2CO_3 and $NaHCO_3$, and, in consequence, the P_H is kept normal until a large part of the bicarbonate has been exhausted. Hence variation in the P_H value is noted as a late change, while the value for the bicarbonate of the plasma changes from the beginning of the introduction of acid. Thus, if acid be added to the blood, owing to increased production in pathological processes or by introduction from without, it would react with the $NaHCO_3$ to form a neutral salt and, consequently, set free some H_2CO_3 . The effect would be a diminution of the denominator of the normal ratio of 1 : 20 with a resultant increase of this value. The increase in P_H would not be proportional to that of the added acid, on account of the buffer action but it would nevertheless tend to show a slight change. If this acid were being added continuously, the hydrogen ion concentration would rise unless some method existed for neutralizing the effect by decreasing the numerator at the same time that the denominator was decreased. Whenever, either by increased rate of CO_2 production or by decomposition of $NaHCO_3$ by acid, the normal ratio of 1 : 20 is increased, the hydrogen ion concentration is proportionally increased. Whether it is the increase in hydrogen ion concentration or the accumulation of CO_2 that is effective, the fact remains that the respiratory center is stimulated so that more rapid ventilation of the lungs follows until the H_2CO_3 is so reduced that the normal

ratio of 1 : 20 is restored. The work of Scott would seem to indicate that CO_2 itself may excite the center quite independently of the H ion concentration of the blood. Boothby reports that the heart output is increased in the effort to rid the body of the excess of CO_2 . To distinguish the stage of acidosis in which the respiratory mechanism no longer keeps the CO_2 concentration of the blood down to the normal value of 1/20 of the bicarbonate and in which the P_H does actually increase, Hasselbalch and Gammeltoft use the term "uncompensated acidosis;" so long as the respiration, in spite of a diminution in the amount of bicarbonate, is able to keep the relations down to normal limits, the condition is known as "compensated acidosis." We may, therefore, define acidosis as any condition in which the proportion between H_2CO_3 and NaHCO_3 becomes greater than 1 : 20; or as Van Slyke defines it, a condition in which the concentration of bicarbonates in the blood is reduced below the normal level. Since the pulmonary epithelium permits the free CO_2 of the blood to diffuse readily through it, it follows that the percentage of CO_2 in the alveolar air must be a measure of the available NaHCO_3 in the blood. As MacLeod expressed it, "To repeat, for this is the fundamental conception of the whole acidosis problem, since C_H remains constant in the blood, the ratio H_2CO_3 : NaHCO_3 must also remain at its normal value of 1/20, and, therefore, if NaHCO_3 declines, H_2CO_3 must decline proportionately, and since this diffuses as CO_2 into the alveolar air, the percentage of this gas in the latter must be proportional to the degree to which foreign acid can be added to the blood without perceptibly changing C_H , in other words it must be proportional to the reserve alkalinity. One other factor must clearly come into play to permit of the smooth operation of the above mechanism, namely, the ratio of pulmonary ventilation must be adapted to the amount of CO_2 that has to be eliminated. This adaptation depends on the respiratory center, the activity of which is preeminently dependent upon the acid base equilibrium in the blood." As the free CO_2 is eliminated the bicarbonate of the blood decomposes, because of the presence of other acid groups in the blood, and the amount that is left indicates the remaining ability of the blood to withstand further addition of foreign acid. "Clearly, therefore, the important thing to measure, in order that we may be enabled to diagnose the incipient stages of acidosis, is the alkaline reserve."

The methods for the determination of the degree of acidosis have been many, applied both to the blood and urine. I select, therefore, those that seem to offer the best criteria from which to judge of this condition of acidosis.

1. VAN SLYKE AND CULLEN'S METHOD FOR CO_2 COMBINING POWER OF PLASMA

This determination is made by the aid of Van Slyke's apparatus. It consists essentially of a 50 c.c. pipet with three-way cocks at top and bottom, and a 1 c.c. scale on the upper stem, divided into 0.02 c.c. divisions. At the bottom the apparatus is connected by a heavy walled rubber tube with

a levelling bulb filled with mercury. The right hand chamber below the lower stop cock serves to draw off the solutions, after the CO_2 has been extracted from them, the other bottom (left hand) connection serving for subsequent release of the vacuum by the entrance of mercury. The apparatus is made of strong glass, in order to stand the weight of mercury without danger of breaking, and is held in a strong screw clamp, the jaws of which are lined with thick pads of rubber. The curved capillary above the upper stop cock is used for removal of solutions from the apparatus. This may, also, be used, for special gas analyses, to connect the apparatus with an absorption pipet. Three hooks or rings are attached to the stand holding the apparatus in such a position that the leveling bulb may be placed in one or other of these positions, as occasion may require. For purposes of the later description of the technic, these positions are known as 1, 2 and 3; the position 1 being such that the lower end of the levelling bulb is even with the upper stop cock of the apparatus; position 2 is such that the lower end of the levelling bulb is even with the lower end of the pipet itself below the lower stop cock; and the position 3 is 80 cm. below position 2. The calibrated upper stem of the pipet is of such diameter that 1 mm. of length corresponds to about 0.01 c.c. By estimating tenths of a 0.02 c.c. division, gas volumes may be read to 0.002 c.c. In order to justify such readings, the apparatus must be accurately calibrated. It is essential that the stop cocks, especially the lower one, shall be held in place so that they can not be forced out by pressure of the mercury. For this purpose rubber bands may be used, but it is more advisable to employ elastic cords of fine wire spirals, as these are stronger and more durable.

Before a determination is made, the entire apparatus, including the capillaries above the upper stop cock, is filled with mercury, a small bottle being placed under the curved capillary to catch any excess of the mercury forced out. To test the apparatus for tightness and freedom from gases, the mercury levelling bulb is lowered to position 3, so that a Torricellian vacuum is obtained, the mercury falling to about the middle of the right hand chamber below the lower stop cock. The levelling bulb is then raised again. If the apparatus is tight and gas-free the mercury will refill it completely and strike the upper cock with a sharp click. In case there is any gas in the apparatus, this serves as a cushion so that the click is not heard and a bubble remains above the mercury. If this is the case, the apparatus must be repeatedly evacuated until the gas has all been removed.

Collection of Specimen for Examination.—For at least an hour before the blood is drawn, the subject should avoid vigorous muscular exertion to prevent any lowering of the bicarbonates of the blood through the production of lactic acid. The blood is drawn from the median basilic vein into a centrifuge tube containing enough powdered potassium oxalate to make about 0.5 per cent. of the weight of the blood drawn. In this process it is advisable to avoid stasis, or when stasis is necessary, to release the ligature as soon as the vein is entered and allow a few seconds for the stagnant blood to pass. While Van Slyke and Cullen state that results of similar significance are

obtained by analysis of either whole blood or plasma, yet, as Macleod points out, there can be little doubt that the whole blood should be used as a part of the alkaline reserve is resident in the corpuscles. However it seems wise to employ plasma and sacrifice a certain degree of accuracy in the method, as this is easier to handle and measure and, in addition, keeps for a longer time without alteration than does whole blood. After collecting the blood, as above, this is centrifuged and the plasma drawn off with a pipet. Place 3 c.c. in a 300 c.c. separatory funnel. To avoid possibility of error while the sample is awaiting analysis, the plasma is now saturated with CO_2 at a definite tension, namely, that of alveolar air containing approximately 5.5 per cent. of CO_2 . The funnel containing the plasma is turned on its side and the air within is displaced by either alveolar air from the lungs of the operator or with 5.5 per cent. CO_2 -air mixture from a tank. In either case, the gas mixture is passed through a bottle containing glass beads, to cool the air and condense the moisture of the breath. When alveolar air is used, as is most common, the operator, without inspiring more deeply than normal, expires as quickly and as completely as possible through the bottle of glass beads and the connecting separatory funnel, the stopper of the funnel being inserted just before the expiration is finished. In order to saturate the plasma with the CO_2 , the stoppered funnel is turned end over end for 2 minutes, the plasma being distributed in a thin layer as completely over the surface of the interior as is possible. The funnel is now placed upright and allowed to stand a few minutes until the fluid has gathered in the bottom of the funnel.

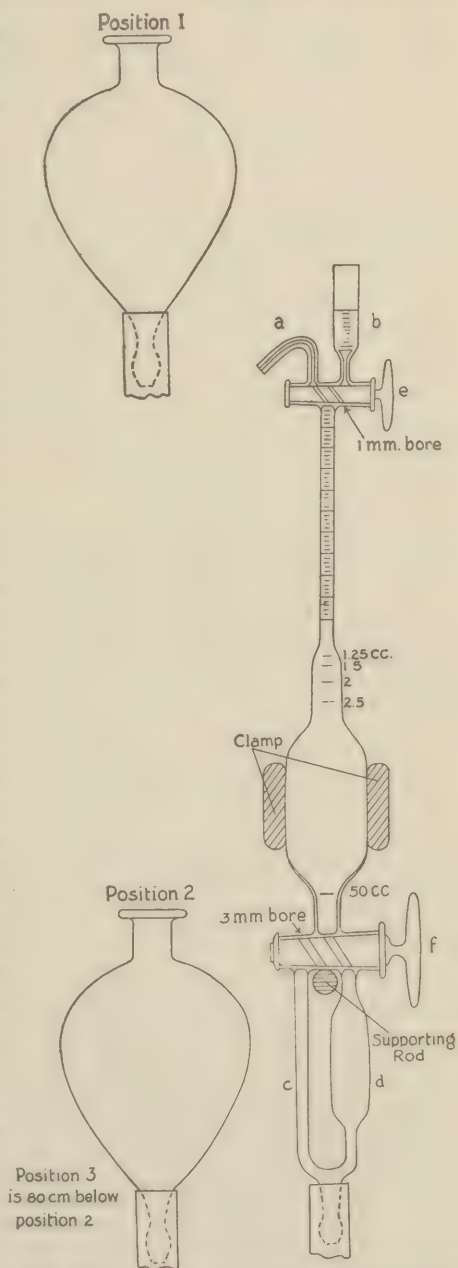


FIG. 120.—Van Slyke Carbon Dioxide Apparatus. (Hawk).

Determination.—The apparatus, including both capillaries above the upper cock, is entirely filled with mercury, and the cup at the top washed free of acid with carbonate-free ammonia, of about 1 per cent. concentration. Not more than 3 or 4 drops of this ammonia are necessary. The 1 c.c. of plasma, necessary for the analysis, is drawn from the funnel by means of an Ostwald pipet and placed in the cup of the apparatus, the tip of the pipet dipping below the solution in the cup. With the mercury bulb at position 2, as mentioned above, and the lower cock in such a position that a connection is established between the pipet and the right hand chamber below the cock, the solution in the cup is admitted to the pipet by opening the upper cock, leaving just enough solution above the upper cock to fill the capillary of the cup. The cup is now washed twice into the pipet with about 0.5 c.c. of water each time, a very small drop of caprylic alcohol (to prevent foaming) is added, and finally 0.5 c.c. of 5 per cent. sulphuric acid is run in. The total volume of the water solution run in must extend exactly to the 2.5 c.c. mark on the apparatus. After the acid has been admitted a drop of mercury is placed in the capillary and allowed to run as far as the cock in order to seal it. Whatever excess of the sulphuric acid remains in the cup is washed out with a little water. After all the solutions are in the pipet, the upper cock being closed and sealed with mercury, the mercury bulb is lowered and hung at position 3, and the mercury in the pipet is allowed to run down to the 50 c.c. mark, producing a Torricellian vacuum in the apparatus. When the mercury (not the water) meniscus has fallen to the 50 c.c. mark, the lower cock is closed and the pipet is removed from the clamp. Equilibrium of the CO_2 between the 2.5 c.c. of water solution and the 47.5 c.c. of free space in the apparatus is obtained by turning the pipet upside down 15 or more times, after which the pipet is replaced in the clamp. By turning the lower cock, the water solution is now allowed to flow from the pipet completely into the right hand lower chamber without, however, allowing any of the gas to follow it. The levelling bulb is then raised in the left hand, while with the right the cock is turned so as to connect the pipet with the lower left hand chamber. The mercury flowing in from this chamber fills the body of the pipet and as much of the calibrated stem at the top as is not occupied by the gas extracted from the solution. A few hundredths of a c.c. of water, which could not be drained off, float on top of the mercury, but this may be disregarded as far as the possibility of reabsorption of CO_2 is concerned. The levelling bulb is then placed at such a level that the gas in the pipet is under atmospheric pressure, that is the surface of the mercury in the levelling bulb should be raised until it is level with the mercury meniscus in the pipet, and, for entire accuracy, raised above the latter meniscus by a distance equal to $1/14$ the height of the column of water above the mercury (this latter precaution introduces, however, such a small error, that it may be ordinarily neglected). The volume of the gas is now read on the scale.

Calculation.—When from plasma, saturated as above described with alveolar air, gases are extracted for analysis, one obtains not only the CO_2 bound as bicarbonate and set free by acidification, but, also, the CO_2 and air

physically dissolved by the plasma and water. These amounts may, of course, be determined by blank analyses or calculated from the known solubility coefficients of the gases. By taking into consideration all corrections necessary, Van Slyke and Cullen have formulated an equation, which permits the determination of the CO_2 bound as bicarbonate by the plasma. This equation is as follows:

$$X = \frac{B}{760} (100.8 - 0.27t)(V - 0.136 + 0.002t)$$

in which B equals the observed barometric pressure; t equals the observed temperature at which the analysis and readings were made; V represents the actual reading, in mm., of the gas in the pipet; X expresses the c.c. of CO_2 reduced to 0° temperature and 760 mm. pressure, which 1 c.c. of plasma will bind as bicarbonate when in equilibrium at 20° with air containing 5.5 per cent. by volume of CO_2 . Van Slyke and Cullen have prepared a table from which the amount of CO_2 bound as bicarbonate by 100 c.c. of plasma may be read, at a glance, in terms of amount of gas shown in the pipet of the apparatus.

By this method, the average normal value for man is found to be 65 volumes per cent. of CO_2 . Austin and Jonas give as the minimum normal figure 60 instead of the 53 which Van Slyke and Cullen stated. From the preceding discussion, it is evident that this value must fall in all conditions associated with an acidosis, so that we find quite low values in diabetes, marked nephritis, most infectious diseases, etc. In severe secondary anemia and in pernicious anemia these values may be low, while in chlorosis the normal values usually obtain.¹

2. VAN SLYKE, STILLMAN, AND CULLEN'S TITRATION METHOD

As the above workers state "In order to titrate accurately the bicarbonate of the blood plasma, it is both theoretically and actually necessary, as a net result of the operation, to transform the bicarbonate into the salt of

¹ Van Slyke and Cullen, *Jour. Biol. Chem.*, 1917, XXX, 289; Van Slyke, *Ibid.*, 347. See, in this connection, Lundsgaard, *Biochem. Ztschr.*, 1912, XLI, 247; Henderson, *Arch. Int. Med.*, 1913, XII, 153; Christiansen, Douglas, and Haldane, *Jour. Physiol.*, 1914, XLVIII, 246; Marriott, *Jour. Biol. Chem.*, 1914, XVIII, 507; Hopkins, *Lancet*, 1914, I, 1589 and 1616; Boothby and Peabody, *Arch. Int. Med.*, 1914, XIII, 497; Fridericia, *Berl. klin. Wchnschr.*, 1914, LI, 1268; Lewis and Barcroft, *Quart. Jour. Med.*, 1915, VIII, 97; Van Slyke, Stillman, and Cullen, *Proc. Soc. Exper. Biol. and Med.*, 1915, XII, 165 and 184; Menten and Crile, *Am. Jour. Physiol.*, 1915, XXXVIII, 225; Peabody, *Arch. Int. Med.*, 1915, XVI, 955; *Am. Jour. Med. Sc.*, 1916, CLI, 184; Marriott, *Arch. Int. Med.*, 1916, XVII, 840; Walker and Frothingham, *Ibid.*, XVIII, 304; Frothingham, *Ibid.*, 717; Macleod, *Jour. Lab. and Clin. Med.*, 1916, II, 54; Hornor, *Boston Med. and Surg. Jour.*, 1916, LXXV, 148; Austin and Jonas, *Am. Jour. Med. Sc.*, 1917, CLIII, 81; de Almeida, *Brazil-Medico*, 1916, XXX, 203; Van Slyke, Stillman, and Cullen, *Jour. Biol. Chem.*, 1917, XXX, 401; Stillman, Van Slyke, Cullen, and Fitz, *Ibid.*, 405; Henderson and Morriss, *Ibid.*, XXXI, 217; McClendon, Shedlov, and Thomson, *Ibid.*, 519; Henderson, *Ibid.*, XXXII, 325; Palmer and Van Slyke, *Ibid.*, 499; Friedman and Jackson, *Arch. Int. Med.*, 1917, XIX, 767; Whitney, *Boston Med. and Surg. Jour.*, 1917, CLXXVII, 225; *Arch. Int. Med.*, 1917, XX, 931; Pearce, *Am. Jour. Physiol.*, 1918, XLV, 550; Scott, *Ibid.*, XLVII, 43; Henderson and Haggard, *Jour. Biol. Chem.*, 1918, XXXIII, 333, 345, 355 and 365; Macleod, *Jour. Lab. and Clin. Med.*, 1919, IV, 315; Moore, *Jour. Physiol.*, 1919, LIII, XXXVI; Parsons, *Ibid.*, 42; Bayliss, *Ibid.*, 163; Haggard and Henderson, *Jour. Biol. Chem.*, 1919, XXXIX,

whatever acid is used in the titration, without altering the normal hydrogen ion concentration of the plasma. If the hydrogen ion concentration is altered, the proteins of the plasma bind as a result either acid or alkali in amounts different from those which they bind *in vivo*. Consequently, under such conditions the proteins act either to increase the acid added in titration to an amount more than equivalent to the bicarbonate, or to depress it below the bicarbonate equivalent." The three requirements of a method, which will permit of the interpretation of the results in terms of plasma bicarbonate are (1) the use of the hydrogen ion concentration of normal blood as the end point; (2) removal of the CO_2 set free by the added acid, and (3) use of an indicator not affected by the plasma proteins. These have been combined in the following method.

In drawing and centrifuging the blood the precautions outlined above in the discussion of the CO_2 Capacity Method, for preventing loss or accumulation of CO_2 and consequent change in the distribution of bicarbonate between corpuscles and plasma, are to be observed. Up to the beginning of the analysis, the blood and plasma are handled exactly as described in the previous method.

Technic.

Two c.c. of the oxalated plasma, saturated with CO_2 , are pipetted into a round-bottomed flask of 150 to 200 c.c. capacity, and 5 c.c. of 0.02 N hydrochloric acid are added (this is about 2 c.c. of acid in excess of the bicarbonate normally present, as this ranges between 0.03 and 0.01 M). In order to remove the CO_2 set free by the acid, the flask is shaken vigorously with a rotary motion for 1 minute, so that the solution is whirled in a thin layer about the inner wall of the flask. The solution is now poured as completely as possible into a 50 c.c. Erlenmeyer flask and the walls of the larger flask are rinsed with 20 c.c. of water, the water being measured in a cylinder and approximately a third used for each of three washings. When the solution and wash fluids, all measuring about 26 c.c., has been transferred to the flask, 0.3 c.c. of a 0.1 per cent. solution of neutral red (dissolved in 50 per cent. alcohol) is added. 0.02 N carbonate-free NaOH is then run in from a buret until the color of the solution matches that of 29 c.c. of a standard phosphate solution, of P_H 7.4, contained in a similar 50 c.c. flask and to which 0.3 c.c. of the neutral red has been added.¹ In place of neutral red, 0.3 c.c. of a 0.04

163; Stadie and Van Slyke, *Ibid.*, 1920, XLI, 191; Haggard, *Ibid.*, XLII, 237; Haggard and Henderson, *Ibid.*, XLV, 189, 199, 209, 215 and 219; Stadie and Van Slyke, *Arch. Int. Med.*, 1920, XXV, 693; Parsons, *Jour. Physiol.*, 1920, LIII, 342; Haldane, *Ibid.*, 1921, LV, 265; Smith, Means and Woodwell, *Jour. Biol. Chem.*, 1921, XLV, 245; Peters, Barr and Rule, *Ibid.*, 489, 537, and 571; Collip, *Ibid.*, XLVI, 61; Henderson, *Ibid.*, 411; Doisy and Eaton, *Ibid.*, XLVII, 377; Haggard and Henderson, *Ibid.*, 421; Taistra, *Ibid.*, XLIX, 479; Chanut, *Ibid.*, 485; Barach, Means and Woodwell, *Ibid.*, 1922, L, 413; Hill *ibid.*, LI, 359; Williams and Sweet, *Jour. Am. Med. Assoc.*, 1922, LXXVIII, 1024; Mellon, Slagle and Acree, *Ibid.*, 1026; Hachen, *Arch. Int. Med.*, 1922, XXIX, 705; Warburg, *Biochem. Jour.*, 1922, XVI, 153; Kennaway and McIntosh, *Ibid.*, 380; Van Slyke, *Jour. Biol. Chem.*, 1922, LII, 495 and 525; Doisy, Eaton and Chouke, *Ibid.*, LIII, 61.

¹ The standard solution of P_H 7.2 and 7.4 may be made according to the directions given in the discussion of the colorimetric method for determining the hydrogen ion concentration of the blood, which follows this. It is important that the 0.02 N NaOH be protected from contact with atmospheric CO_2 and from glass. It should, therefore, be kept in paraffined

per cent. solution of phenolsulphonephthalein (phenol red) may be used as indicator, and gives an end-point slightly more easy to distinguish than that of neutral red. The standard, in the case of phenol red, must be a solution of P_H 7.2, owing to the influence of the proteins on this indicator, as Homer has shown.

It is desirable to use for the titration a micro-buret, although this is not absolutely essential though the results will be a trifle more accurate. A 3 c.c. micro-buret is advised, divided into 0.02 c.c. divisions which are sufficiently separated to permit of readings of 0.01 c.c. The tip is drawn out to a fine point, so that the drops are small, measuring about 0.03 c.c. each. However, in the absence of such a buret, an ordinary one with 0.1 c.c. graduations will suffice. With both indicators, a peculiar phenomenon occurs as the end-point is approached. Each drop appears to change the color past the end-point, but within a few seconds the color shifts back, and it is seen that at least another drop is needed before the real end-point is reached. Consequently, the final color comparison should not be made until at least 30 seconds after the last drop of 0.02 N NaOH has been added.

Calculation.

The number of c.c. of 0.02 N NaOH used in the titration is subtracted from the number required to neutralize to the same indicator 5 c.c. of the 0.02 N HCl used. This number is, of course, approximately 5, but it usually varies slightly from that because of the calibration error of the 5 c.c. pipet used for measuring the acid. Consequently the maximum accuracy is obtained by performing a preliminary titration on 5 c.c. of the acid plus 20 c.c. of distilled water, using the same pipet, indicator, and end-point as in the plasma titration. For example,

$$\begin{array}{ll}
 0.02 \text{ N NaOH} = \text{HCl added} & 5.09 \text{ c.c.} \\
 0.02 \text{ N NaOH taken in titration} & 2.03 \text{ c.c.} \\
 \left. \begin{array}{l} 0.02 \text{ M NaHCO}_3 \text{ in 2 c.c. plasma or} \\ 0.01 \text{ M NaHCO}_3 \text{ in 1 c.c. plasma} \end{array} \right\} & 3.06 \text{ c.c.} \\
 3.06 \div 100 = 0.0306 = \text{molecular concentration of NaHCO}_3 & \\
 \text{in plasma.} & \\
 3.06 \times 22.4 \text{ (or } 0.0306 \times 2240) = 68.5 \text{ volume per cent.} & \\
 \text{CO}_2 \text{ bound as bicarbonate in the plasma.} &
 \end{array}$$

Since the titration result represents c.c. of 0.01 M NaHCO_3 per c.c. of plasma, it is transformed into terms of molecular concentration of NaHCO_3 in the plasma merely by dividing by 100. For the sake of comparison with results of bicarbonate determinations by the CO_2 combining method, the molecular concentration is multiplied by 2,240, in order to obtain results in

bottles and the buret filled with fresh solution as needed. In order to obtain a carbonate-free solution, one may dissolve the NaOH in an equal weight of water. The Na_2CO_3 settles out of such a concentrated alkali solution and the supernatant liquid may be used as the basis of the dilution by taking 5.5 c.c. of this strong solution and diluting to 5 liters, standardizing it against 0.02 N HCl using neutral red as an indicator. In performing this titration it is advisable to run the acid into the alkali, titrating from the yellow alkaline color to the acid red.

terms of CO_2 per 100 c.c. of plasma. According to the gas laws, the amount of CO_2 contained in a M carbonate solution is 22,400 c.c. per liter (measured as CO_2 gas at 0° and 760 mm.) or 2,240 c.c. of gas per 100 c.c. of solution. Hence, multiplying the bicarbonate molecular concentration by 2,240 or multiplying the c.c. of 0.02 N acid used in the titration by 22.4, gives the volume per cent. of bicarbonate CO_2 in the plasma. Inversely, dividing the volume per cent. of CO_2 by 2,240 transforms the CO_2 figures into terms of molecular concentration.

The results with this method agree closely with those of the CO_2 capacity method over the range of bicarbonate concentrations ordinarily encountered in man, even in severe acidosis. Below this range the titration continues to give accurate results, while the CO_2 capacity method gives somewhat higher values. The normal value for the molecular concentration of bicarbonate by this method is 0.0265, corresponding to a CO_2 capacity of 61.6. Stillman recommends that the results be expressed in terms of millimolecular concentration (1 millimolecular = 0.001 molecular).¹

3. METHODS OF DETERMINING HYDROGEN-ION CONCENTRATION

A. Electrometric Method.

This method, known as the gas chain method, is the most accurate one at our command. However, it requires a delicate and expensive physico-chemical apparatus and, which is even more important, considerable special training in such methods before results may be satisfactory. The method consists in measuring the voltage (electric force) set up in a battery of which one electrode is pure hydrogen gas in intimate contact with the solution whose P_{H} it is desired to measure, and the other electrode is of known voltage, the more usual one being the calomel electrode. The development of the electric force at the H electrode is dependent on the concentration of free H ions in the solution, which determines the rate of diffusion between the free H ions in the solution and the H electrode. As everything in the battery is constant, except the concentration of free H ions in the solution, the total electromotive force developed at the H electrode is proportional to the P_{H} of the unknown solution. While, as stated above, this method is one requiring much training in electrochemistry methods, yet it is to be said that the colorimetric methods, which will be discussed in detail, are somewhat less accurate, unless the solutions used are carefully checked by the electrometric method. The literature contains quite a number of reports based on this method, yet it is not one that can find a place in the usual clinical laboratory, so that the details of the method will be omitted.

B. Colorimetric Methods.

This method of determining H ion concentration is based on the fact that each indicator has a characteristic zone of H ion concentration within which its color changes occur. In other words, the exact P_{H} at which indicators

¹ Van Slyke, Stillman, and Cullen, *Jour. Biol. Chem.*, 1919, XXXVIII, 167; Stillman, *Ibid.*, 1919, XXXIX, 261; Haskins and Osgood, *Jour. Lab. and Clin. Med.*, 1920, VI, 37.

change in tint varies with the indicator employed. For convenience in using this method on other fluids than blood, the following table of Clark and Lubs is inserted.

INDICATORS

Chemical Name	Common Name	Color Change	Range P_H
Thymol-sulphon-phthalein.....	Thymol blue	Red-yellow	1.2-2.8
Tetra-brom-phenol-sulphon-phthalein	Brom-phenol blue	Yellow-blue	3.0-4.6
Ortho-carboxy-benzene-azo-dimethyl-aniline.....	Methyl red	Red-yellow	4.4-6.0
Ortho-carboxy-benzene-azo-dipropyl-aniline.....	Propyl red	Red-yellow	4.8-6.4
Dibrom-ortho-cresol-sulphon-phthalein.....	Brom-cresol purple	Yellow-purple	5.2-6.8
Dibrom-thymol-sulphon-phthalein.	Brom-thymol blue	Yellow-blue	6.0-7.6
Phenol-sulphon-phthalein.....	Phenol red	Yellow-red	6.8-8.4
Ortho-cresol-sulphon-phthalein.....	Cresol red	Yellow-red	7.2-8.8
Thymol-sulphon-phthalein (alkaline range).....	Thymol blue	Yellow-blue	8.0-9.6
Ortho-cresol-phthalein.....	Cresol phthalein	Colorless-red	8.2-9.8

Stock solutions of phenol red (or its monosodium salt) may be made of 0.6 per cent. strength. The same strength of cresol red should be prepared. Methyl red and propyl red solutions are prepared by dissolving 0.1 gram in 300 c.c. of alcohol and diluting to 500 c.c. with distilled water. The other indicators should be prepared, as stock, in a strength of 1.2 per cent. The strength, as used in the tests, is usually 0.02 per cent.

Besides the indicators, it is necessary to have a set of "buffer" solutions whose P_H values have been accurately defined by electrometric methods. When these solutions, whose P_H is known, are treated with the indicator, certain colorations appear, which may be compared with those given by the solution to be tested, treated in the same way with the indicator. These buffer solutions must have such well defined compositions that they may be accurately reproduced with such P_H values as have been determined for the same solution by the gas chain method. Sorensen has suggested a set of these solutions, but these have the disadvantage that they are, in part, prepared with acetic acid and, as this is volatile, it becomes a difficult matter to reproduce an exact solution. Other reagents suggested contain an uncertain amount of water of crystallization, which does not permit of accurate determination of amount of salt to add to a solution in order to obtain a definite concentration. For this reason, the writer adopts the set of salts advocated by Clark and Lubs, as it may be accurately standardized and affords a wide range of P_H values.

The various mixtures are made up from the following stock solutions: M/5 potassium chlorid; M/5 acid potassium phosphate; M/5 acid potassium phthalate ($KHC_8H_4O_4$); M/5 boric acid with M/5 potassium chlorid; M/5 sodium hydrate; and M/5 hydrochloric acid. The water used in the

crystallization of the salts and in the preparation of the stock solutions and mixtures should be redistilled. So-called "conductivity water," which is distilled first from acid chromate solution and again from barium hydroxid, is recommended.

M/5 Potassium Chlorid Solution.

The salt should be recrystallized three or four times and dried in an oven at about 120°C. for two days. The solution contains 14.912 grams in 1 liter.

M/5 Acid Potassium Phthalate Solution.

Prepared by the method of Dodge (Jour. Ind. Eng. Chem., 1915, VII, 29) modified as follows: Make up a concentrated KOH solution by dissolving about 60 grams of a high grade sample in about 400 c.c. of water. To this add 50 grams of the commercial resublimed anhydrid of ortho-phthalic acid. Test a cool portion of the solution with phenol phthalein. If the solution is still alkaline, add more phthalic anhydrid; if acid, add more KOH. When roughly adjusted to a slight pink with phenol phthalein add as much more phthalic anhydrid as the solution contains and heat till all is dissolved. Filter while hot, and allow the crystallization to take place slowly. The crystals should be drained with suction and recrystallized at least twice from distilled water. Dry the salt in platinum at 110-115 C. to constant weight. The standard solution should contain 40.828 grams of the salt to 1 liter of the solution. (The phthalic acid in the mother liquors may be recovered by acidifying these and purified by recrystallization.)

M/5 Acid Potassium Phosphate Solution.

A high grade commercial sample of the salt is recrystallized at least 3 times from distilled water and dried in platinum to constant weight at 110-115 C. Solution should contain in 1 liter 27.232 grams. The solution should be distinctly red with methyl red and distinctly blue with brom phenol blue.

M/5 Boric Acid with M/5 Potassium Chlorid.

Boric acid should be recrystallized several times from distilled water. It should be air-dried in thin layers between filter paper and the constancy of weight established by drying small samples in thin layers in a desiccator over CaCl_2 . Purification of KCl is given above. One liter should contain 12.4048 grams of boric acid and 14.912 grams of KCl.

M/5 Sodium Hydroxid Solution.

Dissolve 100 grams NaOH in 100 c.c. distilled water in a Jena or Pyrex Erlenmeyer flask. Cover the mouth of the flask with tin foil and allow the solution to stand over night till the carbonate has mostly settled. Then prepare a filter as follows: Cut a hardened filter paper to fit a Buchner funnel. Treat it with warm strong (1 : 1) NaOH solution. After a few

minutes decant the sodium hydroxid solution and wash the paper first with absolute alcohol, then with dilute alcohol and finally with large quantities of distilled water. Place the paper on the Buchner funnel and apply gentle suction until the greater part of the water has evaporated but do not dry so that the paper curls. Now pour the concentrated alkali upon the middle of the paper, spread it with a glass rod making sure that the paper, under gentle suction, adheres well to the funnel, and draw the solution through with suction. The clear filtrate is now diluted quickly, after rough calculations, to a solution somewhat more concentrated than $N/1$. Withdraw 10 c.c. of this dilution and standardize roughly with an acid solution of known strength, or with a sample of acid potassium phthalate. From this approximate standardization calculate the dilution required to furnish an $M/5$ solution. Make the required dilution with the least possible exposure and pour the solution into a paraffined bottle to which a calibrated 50 c.c. buret and soda-lime guard tube has been attached. The solution should now be most carefully standardized. Proceed as follows: Weigh out accurately on a chemical balance with standardized weights several portions of acid potassium phthalate of about 1.6 grams each. Dissolve in about 20 c.c. distilled water and add 4 drops of phenol phthalein. Pass a stream of CO_2 -free air through the solution and titrate with the alkali till a faint but distinct and permanent pink is developed. It is preferable to use a factor with the solution rather than attempt adjustment to an exact $M/5$ solution.

M/5 Hydrochloric Acid Solution.

Dilute a high grade of HCl to about 20 per cent. and distill. Dilute the distillate to approximately $M/5$ and standardize with the sodium hydroxid solution previously described. If convenient, standardize this, also, by the silver chlorid method.

Compositions of mixtures giving P_H values at 20 C., at intervals of 0.2

Ph	Composition		
1.0	50 c.c. $M/5$ KCl	97.0 c.c. $M/5$ HCl	Dilute to 200 c.c.
1.2	50 c.c. $M/5$ KCl	64.5 c.c. $M/5$ HCl	Dilute to 200 c.c.
1.4	50 c.c. $M/5$ KCl	41.5 c.c. $M/5$ HCl	Dilute to 200 c.c.
1.6	50 c.c. $M/5$ KCl	26.3 c.c. $M/5$ HCl	Dilute to 200 c.c.
1.8	50 c.c. $M/5$ KCl	16.6 c.c. $M/5$ HCl	Dilute to 200 c.c.
2.0	50 c.c. $M/5$ KCl	10.6 c.c. $M/5$ HCl	Dilute to 200 c.c.
2.2	50 c.c. $M/5$ KCl	6.7 c.c. $M/5$ HCl	Dilute to 200 c.c.

Phthalate—HCl Mixtures

2.2	50 c.c. $M/5$ Phthalate	46.70 c.c. $M/5$ HCl	Dilute to 200 c.c.
2.4	50 c.c. $M/5$ Phthalate	39.60 c.c. $M/5$ HCl	Dilute to 200 c.c.
2.6	50 c.c. $M/5$ Phthalate	32.95 c.c. $M/5$ HCl	Dilute to 200 c.c.
2.8	50 c.c. $M/5$ Phthalate	26.42 c.c. $M/5$ HCl	Dilute to 200 c.c.
3.0	50 c.c. $M/5$ Phthalate	20.32 c.c. $M/5$ HCl	Dilute to 200 c.c.
3.2	50 c.c. $M/5$ Phthalate	14.70 c.c. $M/5$ HCl	Dilute to 200 c.c.
3.4	50 c.c. $M/5$ Phthalate	9.90 c.c. $M/5$ HCl	Dilute to 200 c.c.
3.6	50 c.c. $M/5$ Phthalate	5.97 c.c. $M/5$ HCl	Dilute to 200 c.c.
3.8	50 c.c. $M/5$ Phthalate	2.63 c.c. $M/5$ HCl	Dilute to 200 c.c.

Phthalate—NaOH Mixtures

4.0	50 c.c. M/5 Phthalate	0.40 c.c. M/5NaOH	Dilute to 200 c.c.
4.2	50 c.c. M/5 Phthalate	3.70 c.c. M/5NaOH	Dilute to 200 c.c.
4.4	50 c.c. M/5 Phthalate	7.50 c.c. M/5NaOH	Dilute to 200 c.c.
4.6	50 c.c. M/5 Phthalate	12.15 c.c. M/5NaOH	Dilute to 200 c.c.
4.8	50 c.c. M/5 Phthalate	17.70 c.c. M/5NaOH	Dilute to 200 c.c.
5.0	50 c.c. M/5 Phthalate	23.85 c.c. M/5NaOH	Dilute to 200 c.c.
5.2	50 c.c. M/5 Phthalate	29.95 c.c. M/5NaOH	Dilute to 200 c.c.
5.4	50 c.c. M/5 Phthalate	35.45 c.c. M/5NaOH	Dilute to 200 c.c.
5.6	50 c.c. M/5 Phthalate	39.85 c.c. M/5NaOH	Dilute to 200 c.c.
5.8	50 c.c. M/5 Phthalate	43.00 c.c. M/5NaOH	Dilute to 200 c.c.
6.0	50 c.c. M/5 Phthalate	45.45 c.c. M/5NaOH	Dilute to 200 c.c.
6.2	50 c.c. M/5 Phthalate	47.00 c.c. M/5NaOH	Dilute to 200 c.c.

KH₂PO₄—NaOH Mixtures

5.8	50 c.c. KH ₂ PO ₄ M/5	3.72 c.c. M/5NaOH	Dilute to 200 c.c.
6.0	50 c.c. KH ₂ PO ₄ M/5	5.70 c.c. M/5NaOH	Dilute to 200 c.c.
6.2	50 c.c. KH ₂ PO ₄ M/5	8.60 c.c. M/5NaOH	Dilute to 200 c.c.
6.4	50 c.c. KH ₂ PO ₄ M/5	12.60 c.c. M/5NaOH	Dilute to 200 c.c.
6.6	50 c.c. KH ₂ PO ₄ M/5	17.80 c.c. M/5NaOH	Dilute to 200 c.c.
6.8	50 c.c. KH ₂ PO ₄ M/5	23.65 c.c. M/5NaOH	Dilute to 200 c.c.
7.0	50 c.c. KH ₂ PO ₄ M/5	29.63 c.c. M/5NaOH	Dilute to 200 c.c.
7.2	50 c.c. KH ₂ PO ₄ M/5	35.00 c.c. M/5NaOH	Dilute to 200 c.c.
7.4	50 c.c. KH ₂ PO ₄ M/5	39.50 c.c. M/5NaOH	Dilute to 200 c.c.
7.6	50 c.c. KH ₂ PO ₄ M/5	42.80 c.c. M/5NaOH	Dilute to 200 c.c.
7.8	50 c.c. KH ₂ PO ₄ M/5	45.20 c.c. M/5NaOH	Dilute to 200 c.c.
8.0	50 c.c. KH ₂ PO ₄ M/5	46.80 c.c. M/5NaOH	Dilute to 200 c.c.

Boric acid, KCl—NaOH Mixtures

7.8	50 c.c. M/5 H ₃ BO ₃ , M/5 KCl	2.61 c.c. M/5NaOH	Dilute to 200 c.c.
8.0	50 c.c. M/5 H ₃ BO ₃ , M/5 KCl	3.97 c.c. M/5NaOH	Dilute to 200 c.c.
8.2	50 c.c. M/5 H ₃ BO ₃ , M/5 KCl	5.90 c.c. M/5NaOH	Dilute to 200 c.c.
8.4	50 c.c. M/5 H ₃ BO ₃ , M/5 KCl	8.50 c.c. M/5NaOH	Dilute to 200 c.c.
8.6	50 c.c. M/5 H ₃ BO ₃ , M/5 KCl	12.00 c.c. M/5NaOH	Dilute to 200 c.c.
8.8	50 c.c. M/5 H ₃ BO ₃ , M/5 KCl	16.30 c.c. M/5NaOH	Dilute to 200 c.c.
9.0	50 c.c. M/5 H ₃ BO ₃ , M/5 KCl	21.30 c.c. M/5NaOH	Dilute to 200 c.c.
9.2	50 c.c. M/5 H ₃ BO ₃ , M/5 KCl	26.70 c.c. M/5NaOH	Dilute to 200 c.c.
9.4	50 c.c. M/5 H ₃ BO ₃ , M/5 KCl	32.00 c.c. M/5NaOH	Dilute to 200 c.c.
9.6	50 c.c. M/5 H ₃ BO ₃ , M/5 KCl	36.85 c.c. M/5NaOH	Dilute to 200 c.c.
9.8	50 c.c. M/5 H ₃ BO ₃ , M/5 KCl	40.80 c.c. M/5NaOH	Dilute to 200 c.c.
10.0	50 c.c. M/5 H ₃ BO ₃ , M/5 KCl	43.90 c.c. M/5NaOH	Dilute to 200 c.c.

It is important to check the consistency of any particular set of these mixtures by comparing 5.8 and 6.2 phthalate with 5.8 and 6.2 phosphate, using brom cresol purple. Also, 7.8 and 8.0 phosphate should be compared with the corresponding borates using cresol red.

In the method of Levy, Rowntree, and Marriott, which will be immediately discussed in detail, the set of standard solutions is made up from Sorensen's directions as follows: 1/15 M acid or primary potassium phosphate, 9.078 grams of the pure recrystallized salt (KH₂PO₄) are dissolved in freshly distilled water and made up to 1 liter; 1/15 M alkaline or secondary sodium phosphate. The pure recrystallized salt (Na₂HPO₄·12 H₂O) is exposed to the air for from ten days to two weeks, protected from dust. Ten molecules of water of crystallization are given off and a salt of the formula Na₂HPO₄·2H₂O is obtained. 11.876 grams of this is dissolved in freshly distilled water and made up to 1 liter. The solution should give a deep rose red color with phenolphthalein. If only a faint pink color is obtained, the salt is not sufficiently pure. The above two 1/15 M solutions are mixed in the proportions indicated in the table below to obtain the desired P_H.

PH.....	6.4	6.6	6.8	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	8.0	8.2	8.4
KH ₂ PO ₄ c.c.....	73	63	51	37	32	27	23	19	15.8	13.2	11	8.8	5.6	3.2	2.0
NaHPO ₄ .2H ₂ O.....	27	37	49	63	68	73	77	81	84.2	86.8	89	91.2	94.4	95.8	98.0

A. METHOD OF LEVY, ROWNTREE AND MARRIOTT.

The principle of this method is based on the preceding considerations. The color of the standard solutions, to which the indicator has been added, is compared with that given by the solution to be tested after the addition of the indicator. While this method does not use the serum, as such, for the test, as it was believed that the coloring matters and proteins of the blood interfered with the proper colorimetric comparison, yet Homer has been able to obtain results, which agree closely with those of the electro-metric method, by using serum treated with neutral red as the indicator. In the method under discussion, the blood is dropped into collodion sacs and dialyzed for five minutes against 0.8 per cent. sodium chlorid solution. The dialysate, at the end of this period is free from proteins and coloring matter but these may make their appearance in the dialysate in from ten to twenty minutes.

Preparation of Materials.

1. *Standard Color Tubes.*

The solutions are made as previously discussed, with the P_H values ranging from 6.4 to 8.4. Three c.c. of each of the solutions are placed in suitable small test-tubes (100×10 mm., inside measurement). Five drops of an aqueous 0.01 per cent. solution of phenolsulphonephthalein (phenol red) are added to each tube. The tops are sealed off and the tubes are set aside for use as comparison colors. These colors may fade slightly in a month's time, but may still be used for comparison if less indicator is added to the "unknown" solution, as the color quality remains the same.¹

2. *Collodion Sacs.*

One ounce of celloidin (Anthony's negative cotton) is dissolved in 500 c.c. of a mixture of equal quantities of ether and ethyl alcohol. The solid swells up and dissolves with occasional gentle shakings in forty-eight hours. As a small amount of brown sediment may separate out at first, the solution should stand for at least 3 or 4 days, after which time the clear supernatant solution is ready for use. If time permits, one may obtain better sacs from solutions that have "aged" for from 2 to 3 weeks. Small test-tubes (120 by 9 mm. inside measurement) are filled with this solution, inverted, and half the contents poured out. The tubes are then righted, and the collodion allowed to fill the lower half again. A second time it is inverted and rotated

¹ If one does not wish to prepare these solutions for himself, he may obtain accurately standardized ones from Hynson, Wescott & Co., Baltimore, Md.

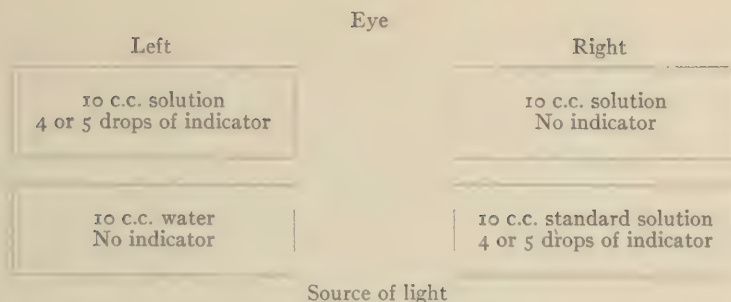
on its vertical axis, the collodion being drained off. Care must be taken to rotate the tube, in order to secure a uniform thickness throughout. The tubes are clamped in the inverted position and allowed to stand for 10 minutes, until the odor of ether disappears. They are then filled with cold water and allowed to soak for 5 minutes. A knife blade is run around the upper rim, so as to loosen the sac from the rim of the test tube, and a few c.c. of water are run down between the sac and the glass of the tube. By gentle pulling, the tube is extracted, after which it is preserved by complete immersion in water, to prevent becoming brittle and impermeable.

3. Salt Solution.

The salt solution, against which the blood is dialyzed, is an 0.8 per cent. solution, which must be free from acids other than carbonic. To determine this, a few c.c. of the salt solution are placed in a Jena test-tube and one or two drops of phenol red are added, whereupon a yellow color appears. On boiling, CO_2 is expelled, and the solution loses its lemon color and takes on a slightly brownish tint. In the absence of this change, other acids are present, and the salt solution is not suitable for use. If, on the other hand, on adding the indicator, pink color at once appears, the solution is alkaline and cannot be used.

Technic.

One to 3 c.c. of clear (non-hemolytic) serum, plasma, whole or defibrinated blood, are run, by means of a blunt pointed pipet, into a dialyzing sac which has been washed inside and outside with salt solution and which has been tested for leaks by filling with salt solution. The sac is lowered into a small test-tube (100×10 mm., inside measurement) containing 3 c.c. of the salt solution, until the fluid on the outside of the sac is as high as on the inside. From 5 to 10 minutes are allowed for dialysis. The collodion sac is removed and 5 drops of the indicator are thoroughly mixed with the dialysate. The tube is then compared with the series of standards until the corresponding color is found, which indicates the P_H of the dialysate. For this color comparison a good light (natural or artificial) and a white background are requisites. Readings must be made immediately. The tube matching most closely is selected and also the tubes on either side of it. These are critically inspected. A color falling between two of the standards may be read, by interpolation, to another decimal place. For this comparison, especially when the original material treated with the indicator is itself colored, the colorimeter of Walpole is essential. This consists of a block of wood with holes bored into the top for the test tubes and openings front and back through which the light may pass and, thus, permit comparison of the color tints. The arrangement of the tubes in this colorimeter may be seen from the following diagram, 10 c.c. of the fluid to be tested being usually employed instead of the 3 c.c. recommended by these workers. The extra tubes are added, in this comparison to equalize the color tints and make the results more accurate.



By this method, oxalated blood from normal individuals gives a dialysate with a P_H varying from 7.4 to 7.6, while that of serum ranges from 7.6 to 7.8. Variations from these figures toward the acid side (lowered P_H) are found in conditions evidenced by acidosis. Even in the more severe acidosis, however, the increase in H ion concentration becomes perceptible only in the final stages. It is, therefore evident that this value must be considered almost a constant and subject to variations under influence of the factors mentioned under the general discussion of the subject of acidosis.¹

B. MARRIOTT'S METHOD FOR ALKALI RESERVE.

As has been stated, the alkali reserve of the plasma is made up of bicarbonates, alkali phosphates, and alkali protein compounds, all of these being

¹ Levy, Rowntree, and Marriott, *Arch. Int. Med.*, 1915, XVI, 389; Levy and Rowntree, *Ibid.*, 1916, XVII, 525. In this connection see Sorensen, *Biochem. Ztschr.*, 1909, XXI, 131 and 201; Walpole, *Biochem. Jour.*, 1910, V, 207; Hasselbalch, *Biochem. Ztschr.*, 1911, XXX, 317; Adler and Blake, *Arch. Int. Med.*, 1911, VII, 479; Sorensen, *Ergeb. Physiol.*, 1912, XII, 393; Hasselbalch and Lundsgaard, *Biochem. Ztschr.*, 1912, XXXVIII, 77; Lundsgaard, *Ibid.*, XLI, 247; Hasselbalch, *Ibid.*, 1913, XLIX, 451; Walpole, *Biochem. Jour.*, 1913, VII, 260; Palmer and Henderson, *Arch. Int. Med.*, 1913, XII, 153; Michaelis, *Die Wasserstoffionkonzentration*, Berlin, 1914; Walpole, *Biochem. Jour.*, 1914, VIII, 628; *Jour. Chem. Soc.*, 1914, CV, 2501 and 2521; Peabody, *Arch. Int. Med.*, 1914, XIV, 236; Milroy, *Quart. Jour. Exper. Physiol.*, 1914, VIII, 141; Hasselbalch and Gammeltoft, *Biochem. Ztschr.*, 1915, LXVIII, 206; Crozier, Rogers and Harrison, *Surg. Gyn. and Obs.*, 1915, XXI, 722; Crile, *Ann. Surg.*, 1915, XXXVII, 257; Lubs and Clark, *Jour. Wash. Acad. Sc.*, 1915, V, 609; Clark and Lubs, *Jour. Infect. Dis.*, 1915, XVII, 160; Marriott, *Arch. Int. Med.*, 1916, XVII, 840; Hurwitz, Meyer, and Ostenberg, *Bull. Johns Hopk. Hosp.*, 1915, XXVII, 16; Lubs and Clark, *Jour. Wash. Acad. Sc.*, 1916, VI, 481; de Corral, *Biochem. Ztschr.*, 1916, LXXII, 1; Scott, *Jour. Lab. and Clin. Med.*, 1916, I, 608; McCleendon, *Jour. Biol. Chem.*, 1916, XXIV, 519; Clark and Lubs, *Ibid.*, XXV, 479; McCleendon and Magoon, *Ibid.*, 669; Parsons, *Jour. Physiol.*, 1917, LI, 440; Clark and Lubs, *Jour. Bacteriol.*, 1917, II, 1, 109, and 191; Sonne and Jarlov, *Hospitalstid.*, 1917, LX, 1247; Cullen, *Jour. Biol. Chem.*, 1917, XXX, 369; Homer, *Biochem. Jour.*, 1917, XI, 283; McCleendon, *Jour. Biol. Chem.*, 1918, XXXIII, 19; Barnett and Chapman, *Jour. A. M. A.*, 1918, LXX, 1062; Macleod, *Jour. Lab. and Clin. Med.*, 1919, IV, 315; Jones, *Jour. Infect. Dis.*, 1919, XXV, 262; Fennel and Fisher, *Ibid.*, 444; Kligler, *Jour. Bacteriol.*, 1919, IV, 35; Stewart, *Am. Jour. Physiol.*, 1919, XLIX, 233; Myers, *Jour. Lab. & Clin. Med.*, 1920, V, 700; Martin, *Biochem. Jour.*, 1920, XIV, 98; Clogne, *Jour. pharm. et chim.*, 1920, XXI, 49; Bessemans, *Jour. Pharm. de Belg.*, 1920, II, 833; Windisch and Dietrich, *Biochem. Ztschr.*, 1920, CII, 141; Walbaum, *Ibid.*, CVII, 219; Parsons, *Jour. Physiol.*, 1920, LIII, CX; Dale and Evans, *Ibid.*, LIV, 167; Bailey, *Jour. Am. Chem. Soc.*, 1920, XLII, 45; Wells, *Ibid.*, 2160; Bunker, *Jour. Biol. Chem.*, 1920, XLI, 11; Koehler, *Ibid.*, 619; Falk and Noyes, *Ibid.*, XLII, 109; Felton, *Ibid.*, 1921, XLVI, 299; McIlvaine, *Ibid.*, XLIX, 183; Michaelis, *Deutsch. med. Wchnschr.*, 1921, XLVII, 465; Zoller, *Jour. Am. Chem. Soc.*, 1921, XLIII, 914; Gillespie, *Jour. Bact.*, 1921, VI, 309; Henderson, Haggard and Coburn, *Jour. Am. Med. Assoc.*, 1921, LXXVII, 424; Acree, Mellon, Avery and Slagle, *Jour. Inf. Dis.*, 1921, XXIX, 7; Evans, *Ibid.*, 1922, XXX, 95; Hirsch and Williams, *Ibid.*, 259; Hirsch and Peters, *Ibid.*, 263; Macleod, *Jour. Lab. & Clin. Med.*, 1922, VII, 369; McCrudden, *Reprint* 730, *Pub. Health Reports*, 1922; Cullen, *Jour. Biol. Chem.*, 1922, LII, 501; Cullen and Hastings, *Ibid.*, 517; Cullen, *Ibid.*, 521; Warburg, *Biochem. Jour.*, 1922, XVI, 153.

present, under normal conditions, in fairly constant amounts. It is due to these constituents that the P_H of the blood is maintained at a constant value. As acid is introduced into the blood, a certain amount of this reserve is drawn upon and the reaction of the blood shifts towards the acid side. While the increased elimination of CO_2 through the lungs may, for a time compensate for this, yet it does not remove the nonvolatile acids nor does this replenish the supply of alkali. Just so long as pulmonary ventilation compensates for the increased production of CO_2 acidosis may not be evident, but sooner or later, the alkali reserve becomes so depleted that the condition may be measured by any of the methods previously discussed.¹

The method of Marriott is a modification of the preceding one of Levy, Rowntree and Marriott. The changes introduced have to do, as far as the materials used in the test are concerned, with the preparation of the solutions used. The phosphate solutions, employed in the previous test, are made up in the same strength by Marriott, but, instead of diluting to 1 liter with water, the phosphates are dissolved in about 800 c.c. of water and, then diluted to 1 liter with 200 c.c. of 0.01 per cent. solution of phenolsulphophthalein, thus obtaining a combination of the standard solution with the dye. These solutions are kept in Non-sol bottles and may contain a small crystal of thymol to prevent growth of molds. The sacks are prepared in the same way for both tests. The salt solution, in Marriott's method, is an 0.8 per cent. solution in water, to which 220 c.c. of 0.01 per cent. phenolsulphophthalein solution are added and the whole made up to 1 liter.

Technic.

The determination should be made, in both these tests, in a room free from both acid and alkaline fumes. Exactly 0.5 c.c. of serum, oxalated plasma, or whole blood, is pipetted into one of the small collodion sacs, which has been previously washed inside and out with the salt solution (in this washing, no part of the sack except the top edge should be touched with the fingers; the sack may be emptied by tipping it with a clean glass rod or with a microscopic slide). This sack is lowered into a small test-tube, approximately 8 mm. internal diameter and 50 mm. long, containing 2 c.c. of the indicator-salt solution. The level of the fluid on the outside of the sack should be at least as high as that on the inside. At the end of 7 minutes the sack is removed and the dialysate transferred to a clean test tube 100 to 140 mm. long and having the same diameter as the tubes containing the phosphate standards. A rapid current of air is bubbled through the solution in order to remove the CO_2 . This is accomplished by means of an atomizer bulb connected with a narrow glass tube drawn out to a capillary point. The air current should be as rapid as possible without blowing liquid out of the test tube. Foaming rarely occurs, but if it should, add a drop of toluol. This blowing is continued for 3 minutes and then the color of the tube is compared with the standard solutions in the manner previously discussed. For convenience of expression the reserve alkalinity is stated as RP_H to differentiate it from the usual P_H .

¹ To avoid confusion it has been suggested that increased and diminished H-ion concentration should be known as acidemia and alkalemia respectively and that diminished and increased alkali reserve should be designated as acidosis and alkalosis.

The serums of a large number of normal adults showed, in every instance, a RP_H value of 8.5 ± 0.05 , provided the subjects were on a general mixed diet. After a fast of sixteen hours, the reading was 8.35. For normal infants, under 1 year of age, a value of RP_H 8.3 was not infrequently noted. Values for the RP_H of from 8.4 to 8.55 correspond to an alveolar CO_2 tension of from 38 to 45 mm., and are to be considered as normal values for adults. Values between 8.0 and 8.3 correspond to an alveolar CO_2 tension of from 28 to 35 mm., and indicate a moderate degree of acidosis. When the value for RP_H is as low as 7.7, corresponding to an alveolar CO_2 tension of 20 mm., the individual is in imminent danger. Marriott states that it is his experience that unless the RP_H of the serum is below 7.9, the acidosis may be successfully combated by dietetic regulation or by administration of alkali by mouth. When it falls below 7.9, intravenous administration of alkali is usually indicated.¹

As a measure of the depletion of the alkaline reserve of the system as a whole the method has been advanced of determining how much alkali may be administered in the form of $NaHCO_3$ to render the urine alkaline. Obviously, when the alkaline reserve is at or near the normal value, very little will be necessary. Sellards and Palmer and Henderson have shown that only 5 grams a day can be taken without making the urine alkaline, when conditions are normal. This quick response to intake of alkalis is seen in the so-called "alkaline tide" of the urine following meals. When the alkaline reserve is seriously depleted, large quantities, even as much as 125 grams of bicarbonate daily may be taken without causing an alkaline reaction of the urine. Macleod states that "for practical purposes it is no doubt the best test of acidosis at present available in routine clinical work."²

(D) Specific Gravity.

The specific gravity of normal blood varies between 1.055 and 1.065, the average being 1.060. Certain variations in this figure are observed de-

¹ Marriott, Arch. Int. Med., 1916, XVII, 840. In this connection, Henderson, Harvey Lectures, 1914-1915, X, 132; Henderson and Palmer, Jour. Biol. Chem., 1914, XVII, 305; Ibid., 1915, XXI, 37; Begun, Herrmann, and Münzer, Biochem. Ztschr., 1915, LXXI, 255; Lang, Biochem. Jour., 1915, IX, 456; Macleod, Jour. Lab. and Clin. Med., 1916, II, 54; Marriott, Jour. A. M. A., 1916, LXVI, 1594; Howland and Marriott, Am. Jour. Dis. Child., 1916, XI, 309; Grulee, Ibid., 1917, XIII, 44; Underhill, Jour. A. M. A., 1917, LXXV-III, 497; McEllroy, Jour. A. M. A., 1918, LXX, 846; Reimann and Bloom, Jour. Biol. Chem., 1918, XXXVI, 211; MacNider, Jour. Exper. Med., 1918, XXVIII, 501 and 517; Schwartz, Levin, and Mahnken, Jour. Cutan. Dis., 1919, XXXVII, 575; Asada, Am. Jour. Physiol., 1919, L, 1; Blackader, Can. Med. Assoc. Jour., 1919, IX, 978; Van Slyke, Stillman, and Cullen, Jour. Biol. Chem., 1919, XXXVIII, 167; McClendon, von Meysenbug, Engstrand, and King, Ibid., 539; Stillman, Ibid., XXXIX, 261; Buell, Ibid., XL, 29; Moore, Lancet, 1919, II, 473; Kuhlmann, Deutsch. Arch. f. klin. Med., 1920, CXXXIII, 346; Collip and Backus, Am. Jour. Physiol., 1920, LI, 551 and 568; Raymond, Ibid., LIII, 109; Sullivan and Stanton, Arch. Int. Med., 1920, XXVI, 41; Carter, Ibid., 310; Tatum, Jour. Biol. Chem., 1920, XLI, 59; Collip, Ibid., 473; Prentice, Lund and Harbo, Ibid., XLIV, 211; Hendrix and Crouter, Ibid., XLV, 51; Hirsch, Jour. Am. Med. Assoc., 1920, LXXV, 1204; Hachen and Isaacs, Ibid., 1624; Willis, Ibid., 1921, LXXVI, 303; Straub and Meier, Biochem. Ztschr., 1921, CXXIV, 259; Hirsch, Jour. Inf. Dis., 1921, XXVIII, 275; Ibid., XXIX, 40; Eggstein, Jour. Lab. & Clin. Med., 1921, VI, 481 and 555; Calvin and Borovsky, Am. Jour. Dis. Child., 1922, XXIII, 493.

² Henderson and Palmer, Jour. Biol. Chem., 1912, XIII, 393; Ibid., XIV, 81; Ibid., XVII, 305; Sellards, Bull. Johns Hopk. Hosp., 1914, XXV, 141; Henderson and Palmer, Jour. Biol. Chem., 1915, XXI, 37; Frothingham, Arch. Int. Med., 1916, XVIII, 717; Sellards, Principles of Acidosis, Harvard Univ. Press, 1917; Macleod, Jour. Lab. and Clin. Med., 1919, IV, 315.

pending on the sex or age of the patient or upon the time and temperature at which the determinations are made.

The most accurate method of determining the specific gravity is, of course, the use of the pycnometer. This method is open to the objection that it requires much more blood than can usually be obtained in routine work. In cases in which bleeding can be resorted to without detriment to the patient this method is the one to use, as it gives the most reliable and accurate results. The writer has used it in many cases of pneumonia, where the withdrawal of a certain amount of blood is often beneficial, and finds the results all that could be desired. Besides the quantity of blood (5 to 50 c.c.) which is required there is also necessary a very accurate chemical balance, else the results will be influenced by the inaccuracies in the weighings.

The technic is as follows: weigh the pycnometer empty, filled with distilled water, and then filled with blood. Care should be taken to have the vessel absolutely dry and clean before weighing it empty and before filling with either water or blood. Subtract the weight of the empty bottle from that of the bottle filled with blood and divide this figure by the difference in weight between the bottle filled with water and the empty bottle. The result will be the specific gravity of the blood, water being taken as unity. We should be careful in this determination to have the temperature of the water the same as that of the blood in order to insure accurate results.

Method of Schmaltz.

This method is a modification of the above and does not give quite as accurate results. It consists in the use of small tubes, which hold about 1/10 c.c. These tubes are constricted at the end to prevent loss of blood and are filled by capillary attraction. The determination is made in the same way as with the pycnometer. More or less manipulative skill is necessary in the handling of these tubes, but the results are sufficiently accurate for most purposes.

The more frequently employed methods of determining the specific gravity are the so-called areometric ones. The principle of these procedures is the determination, by the use of accurate hydrometers, of the specific gravity of a liquid mixture, in the center of which a drop of blood will remain suspended.

Method of Hammerschlag.

This method is a strictly areometric one and consists in the use of a mixture of benzol and chloroform into which a drop of blood is introduced through a capillary tube. If the drop rises in the mixture benzol is added and if it sinks chloroform is used. The point at which the drop of blood remains suspended in the center of the perpendicular axis of the mixture is taken as the one representing the specific gravity of the blood. As the means of estimating the density of this mixture, we employ an accurately graduated hydrometer. It must be remembered that the fluid mixture should be well stirred after the addition of either benzol or of chloroform in order to insure uniform density throughout. As the mixture evaporates rapidly we must work quickly and should confirm our results by a duplicate determina-

tion. A further precaution should be to allow no bubbles of air to adhere to the drop. This is fairly well accomplished by the use of the capillary blood pipet. It is also essential that the temperature of the mixture should not vary to any appreciable extent. This method is simple and, with the precautions mentioned, will yield good clinical results.

The specific gravity of the serum may be tested in this same way, first allowing the blood to coagulate in sealed tubes and then drawing off a drop or two of the separated serum.

Naturally, the specific gravity of the blood is a measure of its concentration and, hence, of its water content. We are, therefore, certain to find variations in this factor under the influence of any physiologic or pathologic changes, which are associated with fluctuations in the volume of blood.

Physiologically, the specific gravity is higher in men than in women and children; is higher in venous than in arterial blood, and is lower after ingestion of large quantities of fluid in the food or after infusions. Clinically, we find that the specific gravity of the blood runs parallel to the number of corpuscles and to the amount of hemoglobin in the red cells. So striking is this ratio that it was formerly used to determine the percentage of hemoglobin in the blood. Any marked alteration in these constituents gives rise to a variation in the specific gravity. Thus, there is observed in anemia in which there is a lowered percentage of hemoglobin, and in those forms of secondary anemia which are characterized more particularly by diminution in the number of cells, a low figure for the specific gravity.

In polycythemia, on the other hand, we find the specific gravity increased as a result of the increased corpuscular content of the blood.

Pathologically, the specific gravity may run between 1,062 and 1,068. An increase is noted in practically all febrile diseases, in those conditions associated with cyanosis, and in disorders leading to obstructive jaundice. In conditions showing marked diuresis, diarrhea, or sweating an increase is likewise observed, but such changes are usually of slight duration, as the blood soon adapts itself to the condition by withdrawing liquid from the tissues to compensate for the loss in the above processes. In nephritis we may find either an increase or a decrease in the specific gravity, depending on the osmotic changes which take place in this disease.

(E) Viscosity of the Blood.

Freshly drawn blood has a greasy feeling, which is replaced by a stickiness as coagulation proceeds. This viscosity or internal resistance of the blood depends, to a large extent, upon the cellular content of the tissue and is distinct from the phenomenon of coagulation.

Many methods have been advanced for the determination of this property of the blood, that of Determann¹ being as clinically accurate and simple as

¹ Münch. med. Wchnschr., 1907, LIV, 1130; Die Viskosität des menschlichen Blutes., Wiesbaden, 1910.

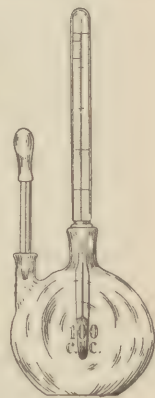


FIG. 121.—Pycnometer.

any. So many uncertain factors influence the viscosity that the writer feels that this determination can add little to our clinical knowledge. As Burton-Opitz¹ says, "Although the results have been gratifying in so far as normal viscosity-values has been established for the human blood, it seems doubtful whether this method will ever be perfected in a way that small variations can be accurately and safely recorded. Nor do the limitations lie wholly in the method. One of the gravest obstacles, encountered in establishing slight differential values, exists in the variability of the viscosity itself."

It has been shown that the degree of viscosity (η) is influenced by cold, withdrawal and application of heat, the former factors causing an increase, while the latter lowers it. Hirsch and Beck demonstrate that the lower the specific gravity of the blood the less marked is its viscosity. These results agree with those that indicate that the lower the specific gravity the lower the number of cellular elements. We may readily see, therefore, why the blood in anemia and leukemia shows such a slight tendency to become sticky or to form rouleaux. The researches of Rotky show that η varies between 5.02 and 5.52 under normal conditions (water being 1), is 1.69 in anemias, 3.34 to 5.58 in nephritis, 13.56 in febrile states and 16.93 in cyanotic conditions. According to Hess, the normal viscosity of male blood ranges between 4.3 and 5.3, while that of female blood varies from 3.9 to 4.9. The normal relation²

$$\frac{\text{Hemoglobin}}{\text{Viscosity}} = 17 \text{ to } 21.$$

(F) Coagulation of the Blood.

It is impossible in this place to discuss the physics and chemistry of the process of coagulation, more than to say that this phenomenon is due to the conversion of the fibrinogen of the plasma into fibrin. This change takes place under the influence of a ferment, thrombase, which is present in the leucocytes and platelets in the form of prothrombase. This latter zymogen, through the influence of calcium compounds, is changed into thrombase, the active agent in bringing about coagulation.

It is occasionally of clinical importance to know the time of coagulation of the blood under certain conditions, as one of the normal processes of this tissue, when outside the vessels, is coagulation. This process takes place normally in from two to eight minutes, depending on several factors, among which are the length of time the blood is in contact with the tissues, the depth of the incision, the pressure with which the blood is expelled, the nature of the vessel into which the blood flows, and the temperature at which coagulation takes place.

¹ Jour. Am. Med. Assn., 1911, LVII, 353.

² Austrian, Bull. Johns Hopkins Hosp., 1911, XXII, 9. See, also, Matsuo, Deutsch. Arch. f. klin. Med., 1912, CVI, 433; Langstroth, Jour. Exper. Med., 1919, XXX, 597 and 607; Weber, Ztschr. f. Biol., 1919, LXX, 211; Harschek, Kolloid. Ztschr., 1920, XXVII, 163; Rothlin, Ztschr. f. klin. Med., 1920, LXXXIX, 233; Bircher, Jour. Lab. & Clin. Med., 1921, VII, 134; Holmes, Jour. Am. Med. Assoc., 1921, LXXVI, 1640; Lyon, Quart. Jour. Med., 1921, XIV, 398; Odaira, Tohoku Jour. Exp. Med., 1921, II, 396.

It has been found that certain variations in the normal coagulability are present at different hours of the day and are observed when blood is drawn from different parts of the body.¹

Dorrance's Method.

This method² is simple and reliable. The instrument consists of an 8-ounce thermos bottle without the silver lining, an aluminium stand and two rubber stoppers, one of which has one hole for the passage of a thermometer, while the other bears four holes each of which is lined with a brass flange. Into each of these holes fits a glass rod $4\frac{1}{2}$ inches long and 8 mm. in diameter. The lower end of each rod is cone-shaped, the flat tip being 4 mm. in diameter. The other end is slightly bulbous to hold the rod in place.

Fill the bottle with water at 98°F. to within an inch of the top. Insert the stopper with thermometer. Scrub the glass rods with soap and water and cleanse them with alcohol and ether. Substitute the stopper with rods for the other, so as to warm them to 98°F. Puncture the ear or finger and wipe away the first two or three drops of blood. Remove the stopper with rods, wipe the latter dry and touch the drop of blood with the tip of each rod in turn, getting as nearly as possible the same amount of blood on each. Note the time and replace the stopper in bottle.

The end-point is determined in three ways: (1) At the end of two and one-half minutes push rod 1 down into the water. All the blood normally falls off the end and breaks up into a fine cloud. At intervals of one minute the other rods in turn are pushed into the water. Some blood falls off but the particles become coarser, the amount remaining on the tip becoming greater until with rod 4 most of the blood remains, if coagulation has occurred. If no coagulation obtains, the test is repeated with larger intervals between the immersions in water. (2) Remove stopper with rods and hold up to the light. The tips will show more or less of a red color depending on the degree

¹ See Cohen, *Arch. Int. Med.*, 1911, VIII, 684 and 820; Howell, *Am. Jour. Physiol.*, 1911, XXIX, 187; *Therap. Gaz.*, 1912, XXXVI, 95; Bordet and Delange, *Ann. de l'Inst. Pasteur*, 1912, XXVI, 657; *Berl. klin. Wchnschr.*, 1914, LI, 497; Barratt, *Jour. Path. and Bacteriol.*, 1913, XVII, 303; Whipple, *Arch. Int. Med.*, 1913, XII, 637; Cannon and Gray, *Am. Jour. Physiol.*, 1914, XXXIV, 232; Cannon and Mendenhall, *Ibid.*, 225, 243 and 251; Gray and Lunt, *Ibid.*, 332; Howell, *Ibid.*, 1914, XXXV, 143 and 474; Pepper and Krumbhaar, *Jour. Infect. Dis.*, 1914, XIV, 476; Fingerhut and Wintz, *Mün. h. med. Wchnschr.*, 1914, LXI, 363; Stuber and Heim, *Ibid.*, 1661; Fonio, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1914, XXVII, 642; *Ibid.*, 1914, XXVIII, 313; Lee and Vincent, *Arch. Int. Med.*, 1914, XIII, 398; *Ibid.*, 1915, XVI, 59; Hurwitz and Drinker, *Jour. Exper. Med.*, 1915, XXI, 401; Drinker, K. R. and C. K., *Am. Jour. Physiol.*, 1915, XXXVI, 305; Mendenhall, *Ibid.*, 1915, XXXVIII, 33; Denny and Minot, *Ibid.*, 233; Bloch, *Arch. d. Mal. du Cœur*, 1915, VIII, 249; Barratt, *Biochem. Jour.*, 1915, IX, 511; Minot, *Jour. Med. Res.*, 1915, XXXIII, 503; Herzfeld and Klinger, *Biochem. Ztschr.*, 1915, LXXI, 391; Klinger, *Cor. Bl. f. Schweiz. Aerzte*, 1915, XLV, 1622; Dale and Walpole, *Biochem. Jour.*, 1916, X, 331; Hekma, *Biochem. Ztschr.*, 1916, LXXIII, 370 and 428; *Ibid.*, LXXVII, 249; Minot, Denny and Davis, *Arch. Int. Med.*, 1916, XVII, 101; Stoldt and Vaughan, *Jour. Lab. & Clin. Med.*, 1916, I, 257; Lenoble, *Bull. de l'Acad. de Med. Paris*, 1916, LXXVI, 365; *Arch. des Mal. du Cœur*, 1916, IX, 539; Bordet, *Reunion soc. belge biol.*, 1919, 1139; Barratt, *Biochem. Jour.*, 1920, XIV, 189; Vines, *Jour. Physiol.*, 1921, LV, 86; Mason, *Jour. Lab. & Clin. Med.*, 1921, VI, 195; Mills, *Jour. Biol. Chem.*, 1921, XLVI, 167; Gratia and Levene, *Ibid.*, 1922, L, 455; Loeb, Fleischer, and Tuttle, *Ibid.*, LI, 461; Pickering and Hewitt, *Biochem. Jour.*, 1922, XV, 710.

² *Am. Jour. Med. Sc.*, 1913, CXLVI, 562. See also, Lyon, *Jour. A. M. A.*, 1916, LXVI, 891.

of coagulation, rod 1 being clear while rod 4 is almost covered with a red clot. (3) Touch the tip of each rod to filter paper. The depth of color of the blot will indicate the degree of clotting. The average coagulation-time by this method is three and one-half to five and one-half minutes.

Rudolf's Method.

This¹ is a modification of the older one of Sabrazès² and applies some of the points of the method of Kottmann.³ It is so simple and clinically reliable that the writer advocates it for general use. Thin glass tubes 1.5 mm. in diameter and about 18 cm. (7 inches) in length are used. A pint Thermos bottle is employed, its ordinary cork being replaced by a triply perforated rubber stopper. In these perforations rest two brass tubes 7 inches long and just large enough in caliber to hold easily the glass blood-tubes. The third perforation contains a thermometer. The apparatus rests on its side in such a way as to prevent its rolling about. The bottle is filled with water at 20°C. and the stopper is inserted.

The finger or lobe of the ear is punctured, the exact time of puncture being noted. Two glass tubes are partially filled from the same drop, the blood being made to run nearly to the far end of each tube, which is placed, as filled, in a brass tube of the thermostat. The protruding ends, the ones at which the blood entered the tubes, are then sealed with a spirit lamp. In about five minutes the first tube filled is drawn out of its holder by the left hand (covered with a glove to diminish the effect of heat of the fingers), is touched with a sharp file, broken across and the broken ends slowly separated. The tube is at once replaced in the thermostat. This technic is carried out with the first tube at intervals of 15 to 30 seconds, until a thread of fibrin appears between the broken ends. The time between the puncture and the appearance of the fibrin is the "coagulation time" of the blood. Tube No. 2 may then be used as a control. Normally the coagulation time averages eight and one-half minutes by this method.

Method of Boggs.

This is a modification of the older method of Russell and Brodie and gives a lower coagulation time than does the preceding method. It is based on the fact that the corpuscles, set in motion by a current of air in a moist chamber, move freely and independently of one another at first but, as coagulation proceeds, clumping of the cells occurs, and finally an elastic radial motion of these cells obtains. This is observed under the low power of the microscope. The writer refers elsewhere for the technic (see cut).

Normally, the coagulation time, as evidenced by the formation of fibrin, is between two and nine minutes.⁴ Anything above nine minutes means

¹ Am. Jour. Med. Sc., 1910, CXL, 807; 1911, CXLII, 481.

² Folia Hæmat., 1904, I, 394.

³ Ztschr. f. klin. Med., 1910, LXIX, 415. For other methods see Rodda, Am. Jour. Dis. Child., 1920, XIX, 269; King and Murray, Jour. Am. Med. Assoc., 1920, LXXIV, 1452; Gram, Ugesk. f. Laeger, 1920, LXXXII, 720; Love, Med. Record, 1920, XCVIII, 436; Inchley, Jour. Pharm. & Exp. Therap., 1921, XVIII, 237; West, Jour. Am. Med. Assoc., 1922, LXXVIII, 1041.

⁴ Carpenter and Gittings (Am. Jour. Dis. Child., 1913, V, 1) show that the average time for children is slightly longer than for adults.

delayed coagulation. The importance of testing this function of the blood is observed more particularly in cases of suspected hemorrhagic diathesis, in which the period of coagulation is remarkably increased. In some cases of hemophilia it requires fifty minutes, while in certain of the purpuras from 10 to 15 or more (Emerson). Likewise, it is customary to test this factor in cases of long-standing jaundice, in which surgical intervention for obstructive lesions of the biliary passages is to be undertaken, as here also the time is much increased. Moreover, we find the coagulation time delayed or imperfect in cases of hemoglobinemia, asphyxia, and general dropsy.¹ Poisoning

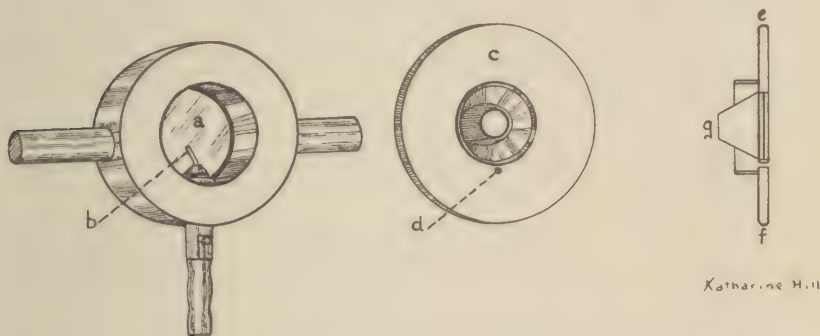


FIG. 122.—Boggs' coagulometer: *a*, moist chamber; *b*, tip of tube through which air passes; *c*, cover which fits over moist chamber and which holds glass cone; *d*, pin-hole for escape of air; *e*, *f*, cross section of cover *c*; *g*, tip of glass cone upon which is placed the drop of blood.

from the bite of certain serpents, such as the cobra, is characterized by a greatly delayed coagulation of the blood as well as by hemolysis. On the other hand, we notice a quicker coagulation in conditions associated with stasis, repeated hemorrhages, transfusion, hunger, and also under the therapeutic use of calcium chlorid and of gelatin.

The formation of fibrin, as the end-product of coagulation, is increased in some cases and diminished in others.² An increase in the amount of fibrin in the blood (hyperinosis) is observed in acute inflammatory processes and in most infectious diseases. Hayem states that the density of the fibrin network, observed when blood is allowed to dry in thick smears on a glass slide, indicates the degree of resisting power of the individual against disease. The largest amounts of fibrin are observed in such conditions as pneumonia³ and acute articular rheumatism, but are seen to a lesser extent in parenchymatous

¹ See Kreiss, Inaug. Dissert., Heidelberg, 1912; Nél, Inaug. Dissert., Berlin, 1912; Landsberg, Biochem. Ztschr., 1913, L, 245; Lee and White, Am. Jour. Med. Sc., 1913, CXLV, 495; Jaffé, Folia Hæmat., 1913, XV, 167; Steiger, Wien. klin. Wchnschr., 1913, XXVI, 1749; Kahn, Am. Jour. Dis. Child., 1916, XI, 103; Hess, Arch. Int. Med., 1916, XVII, 203; Hurwitz and Lucas, *Ibid.*, 543; Corachán and Mones, Siglo. Med., 1919, LXVI, 935 and 960. Schneider, Ztschr. f. d. g. Neur. u. Psychiat., 1919, XIII, 30; Kinsella and Broun, Jour. Am. Med. Assoc., 1920, LXXIV, 1070; Rodda, *Ibid.*, 1920, LXXV, 452; Petren, Beitr. z. klin. Chir., 1920, CXX, 501.

² See Bosworth, Jour. Biol. Chem., 1915, XX, 91; Gram, Jour. Biol. Chem., 1921, XLIX, 279; Foster and Whipple, Am. Jour. Physiol., 1922, LVIII, 365, 379, 393 and 407; McLester, Jour. Am. Med. Assoc., 1922, LXXIX, 17.

³ Dochez (Jour. Exper. Med., 1912, XVI, 693) finds the coagulation time lengthened in acute stage of pneumonia. Anders and Meeker, Jour. A. M. A., 1916, LXVII, 1591; Burns and Young, Am. Jour. Med. Sc., 1917, CLIV, 797.

inflammation, in inflammations of the mucous membranes and the skin, in the febrile stages of chronic suppurations, in hepatitis, influenza, diphtheria, acute gout, and erysipelas. A decrease in the amount of fibrin (hypinosis) may be observed in malignant growths, malarial fever, pernicious anemia, leukemia, and purpura. In parenchymatous nephritis the amount of fibrin is but slightly if at all increased, while in interstitial nephritis the increase may be notable (Da Costa).

(G) Osmotic Pressure and Cryoscopy.

The cryoscopic examination of the blood may be of some importance in the diagnosis of certain conditions, particularly in reference to the sufficiency or insufficiency of the kidneys as regards the elimination of the urinary solids. While this subject has not yielded as much information as was expected of it, a brief discussion seems essential. For a theoretical discussion of osmotic pressure and cryoscopy, I must refer the reader to works on physical chemistry.

By cryoscopy from the Greek *kryos*, frost, and *skopeo*, to see, is meant the determination of the freezing-point of a solution and the referring of this figure to the freezing-point of the solvent, which is regarded as 0. Substances in solution lower the freezing-point of the solvent in direct proportion to their molecular (dissociated or nondissociated) concentration. In determining the osmotic pressure of a solution, and cryoscopy is one of such methods, it is important to remember that the proteins have practically no osmotic value. We have, therefore, in this cryoscopic method a means of ascertaining directly the molecular concentration of the body fluids.

The determination of the freezing-point of the blood is best made by means of the Beckmann apparatus, which may be found in works on physical chemistry. As the osmotic pressure of the serum is equal to that of the plasma or of the whole blood, the serum is generally used for this determination. Withdraw from a vein, preferably the median basilic, about 30 c.c. of blood and allow it to coagulate in a clean closed vessel. Place the serum in the freezing tube of the Beckmann apparatus, adjust the thermometer and stirring rods, and proceed as directed elsewhere.

The freezing-point of the blood is usually designated as the small Greek delta (δ), while that of the urine is given the sign Δ . Normally, blood freezes

at $-0.56^{\circ}\text{C}.$, distilled water freezing at 0. This point is subject to more or less physiologic variation depending upon the changes which occur in the

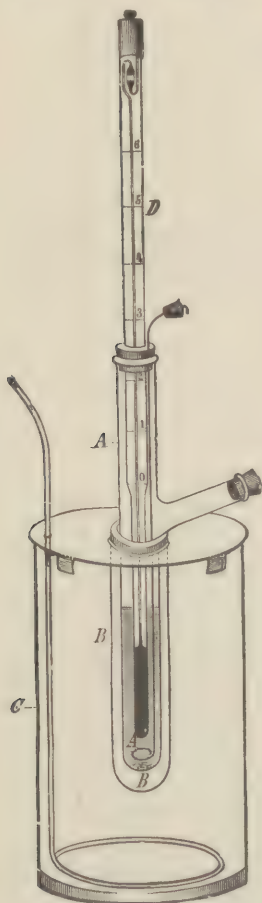


FIG. 123.—Beckmann apparatus. (Long.)

blood after meals, exercise, baths, etc. This physiologic variation is transient and slight because the kidneys soon regulate the osmotic pressure (molecular concentration) of the blood by withdrawal of the constituents which have caused the abnormal tension. This close relationship between the kidneys and the blood is of great importance in the study of renal insufficiency, as many products of metabolic activity remain in the blood in cases of renal disturbance. This is more particularly true of the inorganic constituents of the blood, as the organic elements do not materially affect its osmotic proportions. It is in those cases of renal insufficiency with a tendency to uremia that the most is to be expected from the cryoscopic examination of the blood, yet we find that Schoenborn,¹ in the study of 88 cases, observed practically normal figures for the cryoscopic point of the blood. Engelmann,² on the other hand, reported a series of 36 cases in which the freezing-point averaged -0.664°C . It is true that in certain surgical conditions of the kidneys a parallelism is noticed between the lowering of the freezing-point and the development of the uremic symptoms. In cyanotic conditions of any origin whatever we find the cryoscopic point lower than the normal, owing to the fact that the CO_2 is increased in amount in such cases. If the CO_2 be driven off, the freezing-point becomes normal. Hence we see why, in certain cases of uncompensated cardiac disease, the freezing-point has frequently been reported much lower than the normal standard. There is practically no disease of the blood itself about which a cryoscopic study will furnish any information.

From these considerations, as well as from many others, which I have not enumerated, the study of the cryoscopy of the blood does not seem to the writer of sufficient importance to warrant its adoption as a routine method of blood examination. It is, certainly, of no value whatever to the general practitioner who does not have access to a thoroughly equipped laboratory. The time consumed in such examinations would be much better spent in a more thorough study of the fresh and of the stained specimens. While cryoscopy may give some idea of the osmotic activity of the kidneys, it adds to blood examinations a slightly enlarged laboratory record, whose interpretation is a matter of more or less difficulty and whose results rarely ever give any information, beyond that which may be more easily and more readily learned by other methods. It is a purely theoretical method of following the metabolic activity of the system and reveals no signs of an oncoming uremia nor does it show us anything regarding the results of therapeutic measures.

Naturally, variations in the osmotic pressure of the blood are of importance in the study of conditions associated with effusions into the various serous cavities, as such exudations are markedly influenced by the molecular concentration of the blood. For a discussion of such phases of the subject I must refer the reader to works on pathology.

¹ Wiesbaden 1904.

² Mitt., a. d. Grenzg. d. Med. u. Chir., 1903, XII, 396. See, also, Krotoszyner and Hartman, Jour. A. M. A., 1913, LX, 188; Ibid., 1915, LXV, 1788; Lippmann, Zentralbl. f. inn. Med., 1920, XLI, 41; Collip, Jour. Biol. Chem., 1920, XLII, 207, 221 and 227.

(H) Electric Conductivity.

In the effort to enlarge the scope of blood examinations, the clinician and, more particularly, the laboratory worker have taken advantage of everything offered for the furtherance of their aim. In so doing they have often over-stepped themselves and complicated the examinations by useless additions to their technic.

By electric conductivity is meant the reciprocal of the resistance, which a certain amount of solution offers to the passage of an electric current of known strength between two platinum electrodes of given size and a given distance apart. This method is merely a measure of the number of electrolytes (both dissociated and nondissociated) in solution and is not affected by the nonelectrolytic organic substances of the blood, although, according to Hardy,¹ the proteins move to one or the other pole of the battery depending on the reaction of the fluid. The method usually adopted for estimating this factor is that of Kohlrausch, the resistance being balanced on a Wheatstone bridge against a rheostat, the point of equilibrium being determined by means of a telephone attachment.

As this determination can give us no information regarding the retention of organic products in the system, it is of comparatively little value in the study of metabolic or of blood diseases. It is in those conditions which are associated with retention of inorganic constituents that this method should be of value, and yet we find, according to the work of various authors, that in advanced nephritis, in which the retention of chlorids has been assumed, the conductivity of the serum is not increased to any extent, although the freezing-point is lower than the normal standards. This proves, it seems to the writer, that in such cases the substances capable of increasing the conductivity—that is, the chlorids—are either not retained in the blood or are in such a combination as not to influence the conductivity of this tissue. We must, therefore, assume that the lowering of the freezing-point observed in such conditions is due more to the organic than to the inorganic constituents of the blood. As this determination is, in the writer's opinion, of so little practical value in blood work, a detailed description of the methods and results of observations will not be taken up.

(6) Chemical Properties.

The chemical examination of the blood resolves itself into a consideration of the composition of the whole blood, of the plasma, of the serum, and of the various cellular elements. It is evident that the composition of the whole blood will depend upon the relations in which the single constituents of the blood stand to one another.² Naturally, physiologic influences are of great importance in the consideration of the composition of the fluid portion, while the cellular elements are less affected by these changes. In view of the recent work on opsonins and immunity, we must conclude that the chemical examination of the fluid elements of the blood may yield much important information as soon as proper methods of research are evolved.

¹ Proc. Royal Soc., 1900, LXVI, 95. See Collip, Jour. Biol. Chem., 1920, XLII, 213.

² See Bönninger, Ztschr. f. exp. Path. u. Therap., 1912, XI, 1; also, Veil, Deutsch. Arch. f. klin. Med., 1914, CXIII, 226; Löwy, Deutsch. Arch. f. klin. Med., 1915, CXVII, 79.

Normally, the composition of the cellular elements remains almost constant and is but slightly affected by the varying composition of the fluid portions. As is well known, the corpuscles act more or less like semipermeable membranes, although Kahlenberg would have us believe that no such a condition is possible. At any rate, the membrane surrounding the erythrocyte allows the passage of certain inorganic and organic constituents of the plasma into the cells and permits the back-passage of certain constituents of the cells into the plasma. This question is intimately related to the subject of hemolysis, which will be discussed in detail later. It is true that a many-sided exchange exists between the red cells and the hypertonic plasma in which they float, such an exchange accounting very often for the alterations in the volume of the corpuscles and frequently being the cause of abnormally developed cells. This exchange is subject to certain restrictions. Thus we find that the corpuscles show a very high content in potassium salts and a very low sodium content, while the reverse conditions obtain in the serum. If free exchange took place between the cells and serum no such conditions could exist. It is known that potassium salts are closely associated with phenomena of growth and hence it might be possible to prove that cells, which are morphologically the older, show a less potassium content than do the newly developing ones.

It is evident that a quantitative analysis of the blood can be of only comparative value as far as the blood as a whole is concerned. We should ascertain on one side the relationship of the plasma and blood-corpuscles to each other, and on the other side the composition of each of these two chief constituents. As there are many difficulties in the way of such determinations, we will not go into detail regarding the chemical composition of the different portions of the blood, but will discuss the blood as a whole. According to C. Schmidt, the composition of the blood is as outlined in the following table.

	Man's.	Woman's.
Corpuscles	513.02	396.24
Water	349.690	272.560
Solids	163.330	123.680
Hemoglobin, proteins, and other organic bodies . . .	159.590	120.130
Inorganic bodies	3.740	3.550
K ₂ O	1.586	1.412
Na ₂ O	0.241	0.648
CaO	0.320	0.485
MgO		
Fe ₂ O ₃		
Cl	0.898	0.362
P ₂ O ₅	0.695	0.643

	Man's.	Woman's.
Serum	486.98	603.76
Water	439.020	551.990
Solids	47.960	51.770
Protein and other organic bodies	43.820	46.700
Inorganic bodies	4.140	5.070
K ₂ O	0.153	0.200
Na ₂ O	1.661	1.916
CaO	0.533	0.608
MgO		
Cl	1.722	0.144
P ₂ O ₅	0.071	2.202

This table, while giving the composition of the whole blood, may readily serve as one from which the composition of the corpuscles and serum may be obtained by reducing the constituents to parts per 1,000 of either corpuscles or serum. It is beyond the scope of this work to discuss the chemical properties of the blood or of its constituents in detail, but certain points which have clinical interest must be taken up.

The large excess of chlorin in the serum of man as compared with that of woman and the excess of phosphoric acid in the serum of woman as compared with that of man is noteworthy. These variations may later be shown to have a great influence upon sexual differences as regards metabolism.¹

(A). Total Solids.

The determination of the total solids and, hence, of the water content of the blood is often of importance, especially in cases of anemia.² The method is as follows: Allow about 1 c.c. of blood to flow onto one of two previously weighed matched watch-glasses. Cover this with its mate and weigh them while in the moist condition. Separate the glasses and place them in a desiccator over CaCl₂ or H₂SO₄ for twenty-four hours, at the end of which time weigh them as before. The blood should have dried to a hard glassy mass in this time. The loss of weight will represent the water of the blood taken, from which amount the percentage may be calculated. Gumprecht and Stintzing and Biernacki advise the use of higher than room temperature, the former using 67°C. in an incubator, while the latter employs a heat of 100 to 120°C. Drying by these latter methods is often associated with loss; hence the writer is accustomed to use room temperature (about 20°C.).

Working in this way, many have found the dry residue (total solids) of the blood to be from 21 to 22.5 per cent., the water content being 77.5 to 79 per cent. This figure shows marked variation under the influence of such processes as diarrhea, excessive sweating, and exudation into serous cavities. In anemia the solids are much reduced, while in leukemia they are increased.

¹ See Gettler and Baker (Jour. Biol. Chem., 1916, XXV, 211) for extensive analysis of the blood of 30 normal cases. Also Gettler and George, Jour. A. M. A., 1918, LXXI, 2033. Hammett (Jour. Biol. Chem., 1920, XLI, 599) reports an extensive study of the normal variations in the nitrogenous constituents and sugar of the blood.

² See B. sch, Ztschr. f. exper. Path. u. Therap., 1913, XIV, 225; Peters and Rubnitz Arch. Int. Med., 1920, XXVI, 561.

(B) Blood Pigments.**Hemoglobin.**

The normal color of the blood is due to the presence of the pigments, hemoglobin and oxyhemoglobin. Usually the former is not large in amount, while the latter is the predominant factor in the color. In blood after asphyxia we find a mixture of hemoglobin, pseudohemoglobin, and parhemoglobin; in arterial blood large amounts of oxyhemoglobin, and in venous blood a mixture of hemoglobin and oxyhemoglobin.

Hemoglobin belongs to the class of bodies known as "chromoproteins" and, owing to its power of combining with various gases and thus aiding in the gaseous exchange of the body, may be styled a "respiratory protein." It is easily decomposed into a protein, globin (about 96 per cent.), and a pigment, hemochromogen (4 per cent.), which contains iron and is easily changed in the presence of oxygen into hematin. The iron content of hemoglobin is the portion which enables this pigment to exert its peculiar vital power of oxygen transference.¹

The amount of hemoglobin in normal blood is variable, depending on the age of the subject examined.² Normally, 100 c.c. of adult human blood contain from 16 to 17 grams of hemoglobin (the average being about 16.92), which amount is not a constant factor at all ages. From the following table of Leichtenstern it will be seen that a definite age curve exists for this substance, although his figures are lower than those obtained by more exact methods. Altitude also influences the amount of hemoglobin to a certain extent. Thus, the oxygen capacity of the blood at sea level varies between 17 and 18.7 per cent., while on Pike's Peak it is about 27.4 per cent. As a general rule, for every 100 mm. fall in the atmospheric pressure there is an average rise of about 10 per cent. in hemoglobin.

<i>Age.</i>	<i>Grams per 100 c.c. of blood.</i>
1 to 4 days,	19.329 to 21.160
8 to 14 days,	17.869 to 16.124
8 to 20 weeks,	15.362 to 12.928
6 months to 5 years,	10.971 to 11.373
5 to 15 years,	11.151 to 11.796
15 to 25 years,	13.034 to 13.870
25 to 45 years,	14.727 to 15.013
45 to 60 years,	12.484 to 13.150
Over 60 years,	14.790

Under certain conditions the hemoglobin is dissolved out from the red cells, leaving only their stromata behind. This gives rise to the condition known as hemoglobinemia which will be discussed in a later section. Accord-

¹ See Barcroft (*Respiratory Function of the Blood*, Cambridge, 1914) and Macleod, *Physiology and Biochemistry in Modern Medicine*, St. Louis, 1918.

² See Williamson (*Arch. Int. Med.*, 1916, XVIII, 505) for a spectrophotometric analysis of 919 cases of varying ages. His figures show that between the ages of 16 and 60 there is a marked difference between the two sexes, this difference growing less after the 60th year. The variations in the normal values for hemoglobin are greatest from birth to the 16th year. See also, Appleton, *Jour. Biol. Chem.*, 1918, XXXIV, 369.

ing as hemoglobin is free or combined with certain gases, we have in the blood various derivatives of this pigment, each one of which has certain characteristics and a definite clinical importance. These derivatives are recognized by qualitative tests, especially by spectroscopic methods, and will be presented here; while the important tests used for the recognition of hemoglobin, either in the fresh state or as dried stains, will be taken up under the medicolegal discussion.¹

Hemoglobin also called reduced hemoglobin, is much more soluble than oxyhemoglobin, its solution in water being more violet or purplish than one of oxyhemoglobin of the same concentration. Such solutions of hemoglobin absorb the blue and violet rays of the spectrum to a less marked degree than do those of oxyhemoglobin, but they strongly absorb the rays lying between *C* and *D*. In proper dilution a solution of hemoglobin shows a spectrum with one broad not clearly defined band between *D* and *E*, lying toward the red end of the spectrum a little over the Fraunhofer line *D* (see plate).

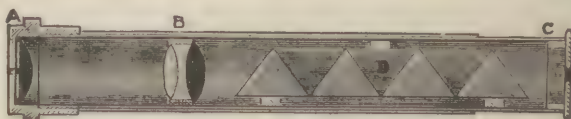


FIG. 124.—Direct-vision spectroscope.

Pseudohemoglobin.

According to Ludwig and Siegfried, blood, reduced by hyposulphites or by a stream of hydrogen to such an extent that the spectrum of oxyhemoglobin disappears, yields large amounts of oxygen when exposed to a vacuum. This loose combination of hemoglobin and oxygen, which gives the spectrum of hemoglobin, is called pseudohemoglobin. Hammarsten considers it an intermediate body between hemoglobin and oxyhemoglobin.

Oxyhemoglobin.

Oxyhemoglobin, also called hematoglobulin, is a molecular combination of hemoglobin and oxygen. The ability of hemoglobin to take up oxygen is a function of its iron content. When this factor is calculated as 0.33–0.40 per cent., 1 atom of iron in the hemoglobin molecule corresponds to two atoms (one molecule) of oxygen. This combination is a loose one and hence the quantity taken up will depend upon the partial pressure of the oxygen. This oxygen is set free when the oxygen pressure is reduced, thus giving rise to the characteristic property of oxygen transference. As Pflüger has shown, oxyhemoglobin may, when it is gradually oxidized, act as an “ozone exciter” by the decomposition of neutral oxygen into the atomic form. It may also act as an “ozone transmitter” as in certain tests to be outlined later.²

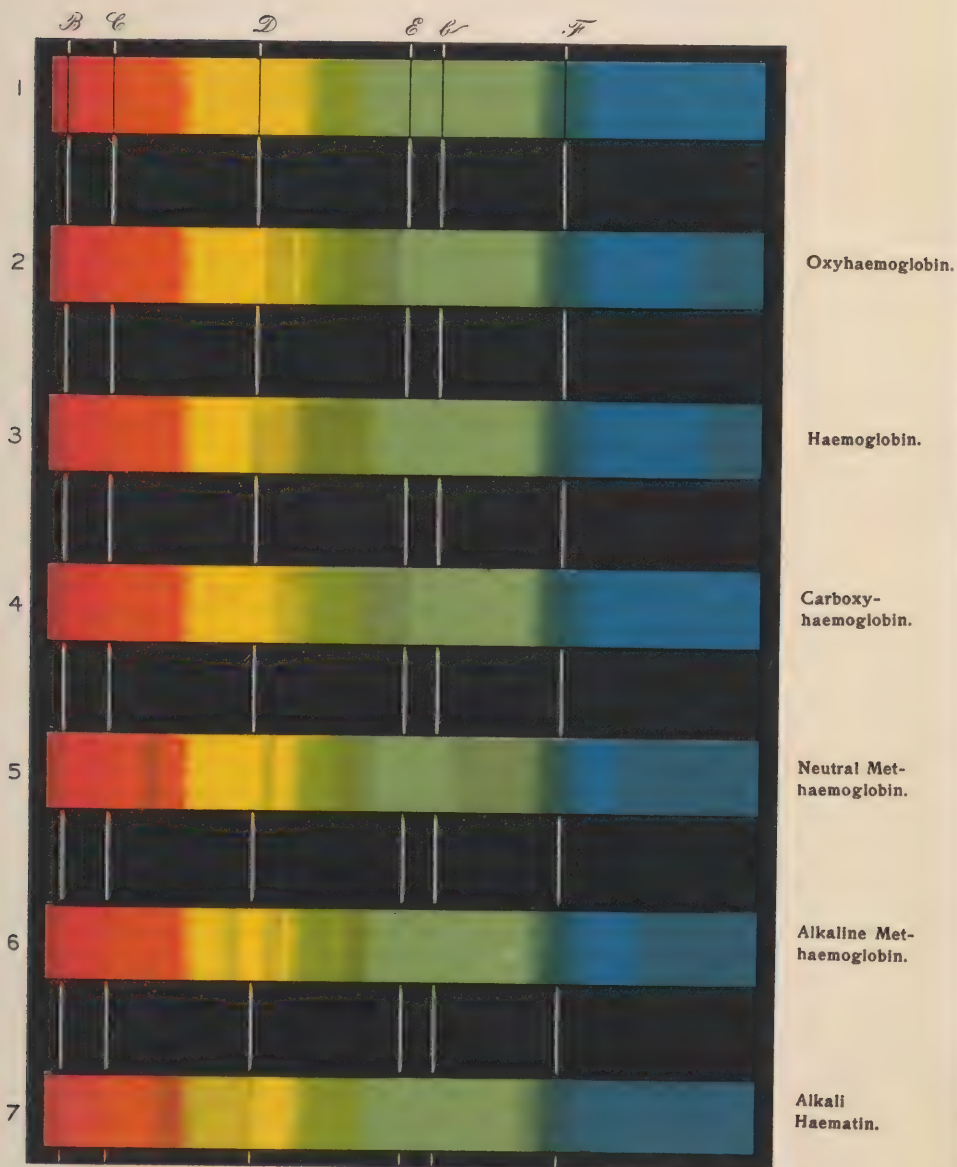
A dilute solution of oxyhemoglobin or of arterial blood shows a spectrum with two absorption bands between the Fraunhofer lines *D* and *E*. One band, α , is narrower, but darker and sharper and lies on the line *D*, the other,

¹ See Maestre and Lecha-Marzo, Arch. internat. de méd. leg., 1914, V, 49.

² See McClendon, Jour. Biol. Chem., 1915, XXI, 275.

Absorption Spectra.

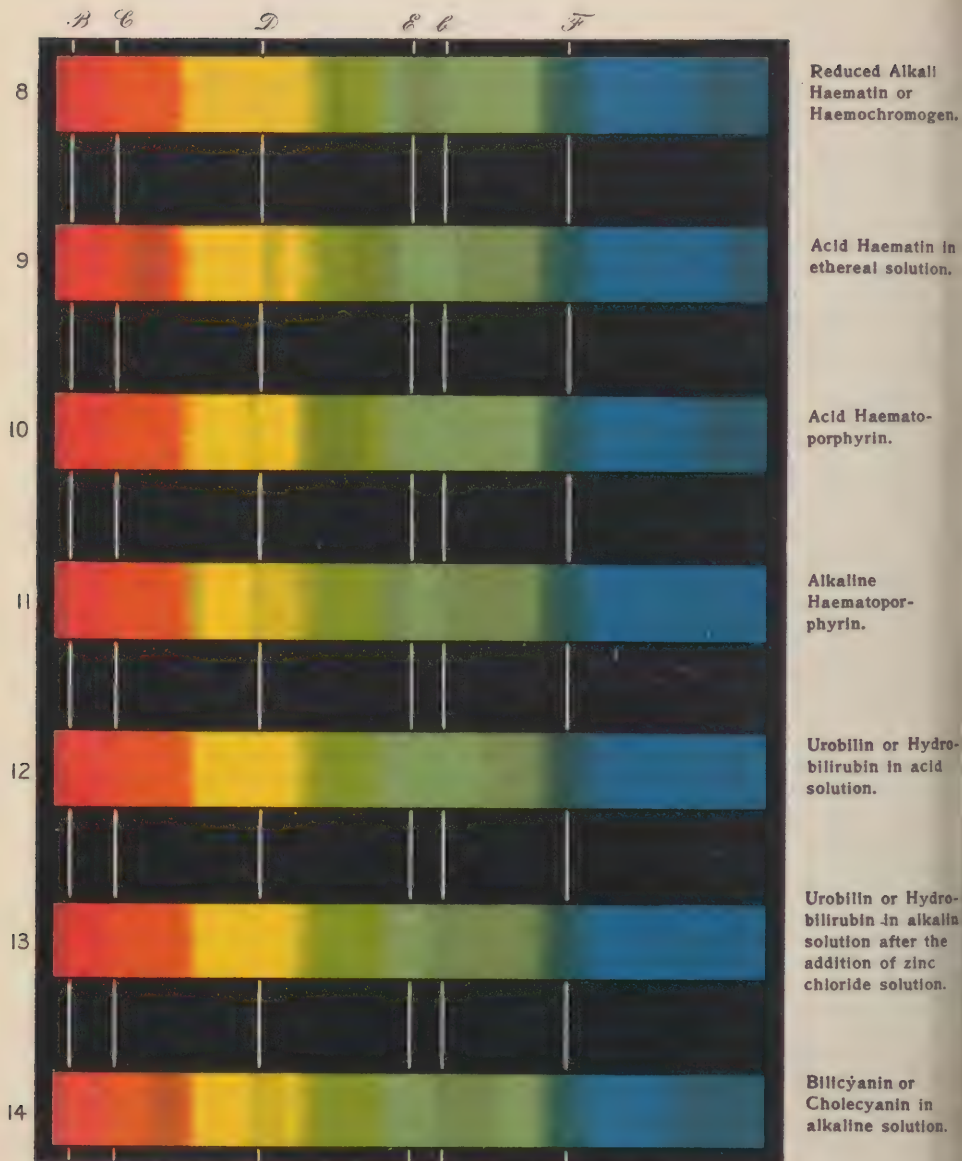
PLATE XV.



(FROM HAWKS "PHYSIOLOGICAL CHEMISTRY")

Absorption Spectra.

PLATE XVI.



(FROM HAWKS "PHYSIOLOGICAL CHEMISTRY")

β , is broader, less defined and less dark and lies at *E*. As the dilution becomes weaker the band β first disappears. By increased concentration the two bands become broader, the space between them smaller, and the blue and violet parts of the spectrum darkened. Other substances may give this same absorption spectrum, but oxyhemoglobin may be differentiated by its behavior toward reducing agents, such as ammonium sulphid or Stokes' solution of ammoniacal ferrotartrate (see plate).

Methemoglobin.

This pigment is closely related to oxyhemoglobin, as it contains the same amount of oxygen and is isomeric with it. The oxygen is, however, not in loose combination and cannot, therefore, be utilized by the system. This coloring matter is formed by the spontaneous decomposition of blood, as observed in hemorrhagic transudates and cystic fluids, and occurs also in cases of poisoning with potassium permanganate, potassium ferricyanid, chlorates, nitrites, nitrobenzol, acetanilid, antipyrin, turpentine, sulphonal, and arsenic, and in cases of cyanosis with diarrhea.

According to Jäderholm and Bertin-Sans, the absorption spectrum of methemoglobin, in aqueous or slightly acidified solution, is similar to that of acid hematin (see below), but is easily distinguished from the latter by the readiness with which it turns into that of hemoglobin on treatment with alkali and a reducing substance. Hematin, under the same conditions, gives the spectrum of an alkaline solution of hemochromogen¹ (Hammarstein). Methemoglobin, in alkaline solution shows two absorption bands, which are like those of oxyhemoglobin, but differ from them in that the band nearer *E* is stronger than the one at *D*. A third fainter band may be observed, according to Hammarsten, lying between *C* and *D*.

Quantitative determination.

Method of Stadie.

This method² depends on the fact that both hemoglobin and methemoglobin are changed quantitatively to cyanhemoglobin by dilute solutions of potassium cyanid. The color of this latter compound is a brilliant orange-red and is very suitable for colorimetric comparison with a standard solution of the same derivative.

Two c.c. of oxalated whole blood are placed in a 100 c.c. flask and 50 c.c. of water are added, which effect hemolysis in a few seconds. 0.5 c.c. of a 0.1 M (3 per cent.) solution of potassium ferricyanid is added, and the flask allowed to stand for 15 to 20 minutes. Five c.c. of a 0.1 per cent. potassium cyanid solution are now added, bringing about an immediate change to cyanhemoglobin. Water is added to the mark and the solution compared with a standard of known strength in the colorimeter. The result is the hemoglobin plus methemoglobin, which is expressed as grams of "total hemoglobin" per 100 c.c. of blood.

¹ Halliburton and Rosenheim (Biochem. Jour., 1919, XIII, 195) suggest that the name of this pigment be changed to "reduced hematin."

² Stadie, Jour. Biol. Chem., 1920, XLII, 237; Jour. Exp. Med., 1921, XXXIII, 627. McEllroy (Jour. Biol. Chem., 1920, XLII, 297) has devised a method for this determination which is very similar.

Preparation of Standard.—The standard solution is prepared from fresh whole oxalated or defibrinated blood which is known to contain no methemoglobin. The hemoglobin content (grams per 100 c.c.) is determined by the method of Van Slyke (discussed under determination of hemoglobin, page 478). Five hundred c.c. of standard are made by placing 10 c.c. of the blood in a 500 c.c. flask, hemolyzing with about 300 c.c. of water, and adding 2.5 c.c. of the potassium ferricyanid solution. After 20 minutes, 25 c.c. of the potassium cyanid solution are added and the mixture diluted to the mark. The blood pigment value of the solution is known from the gasometric determination, and the unknown may be compared directly with it or suitable dilutions of the standard may be made.

Factors for Calculating Results from Analysis of 2 c.c. of Blood Saturated with Air

Temperature	Air physically dissolved by 2 c.c. of blood. Subtract from gas volume to obtain corrected gas volume representing O ₂ set free from hemoglobin	Factor by which corrected gas volume is multiplied in order to give hemoglobin in 100 c.c. of blood
°C.	c.c.	gm.
15	0.037	$34.7 \times \frac{B}{760}$
16	0.036	$34.6 \times \frac{B}{760}$
17	0.036	$34.3 \times \frac{B}{760}$
18	0.035	$34.2 \times \frac{B}{760}$
19	0.035	$34.0 \times \frac{B}{760}$
20	0.034	$33.9 \times \frac{B}{760}$
21	0.033	$33.7 \times \frac{B}{760}$
22	0.033	$33.5 \times \frac{B}{760}$
23	0.032	$33.4 \times \frac{B}{760}$
24	0.032	$33.1 \times \frac{B}{760}$
25	0.031	$33.0 \times \frac{B}{760}$
26	0.030	$32.9 \times \frac{B}{760}$
27	0.030	$32.6 \times \frac{B}{760}$
28	0.029	$32.5 \times \frac{B}{760}$
29	0.029	$32.3 \times \frac{B}{760}$
30	0.028	$32.1 \times \frac{B}{760}$

A small portion (4 to 5 c.c.) of the original blood is aerated in a funnel and its total oxygen capacity determined by the method of Van Slyke, as given on page 478. As the hemoglobin is practically 100 per cent. saturated under these conditions, the oxygen capacity corresponds to the amount of hemoglobin, and, by dividing by 1.34 (the volume of oxygen combined with 1 gram of hemoglobin) we obtain the grams of hemoglobin per 100 c.c. of blood. For convenience of calculation the factors for the conversion of c.c. of gas combined with 2 c.c. of blood into grams of hemoglobin per 100 c.c. of blood are given in the table above (modified from Van Slyke's table as given on page 480).

Calculation.—This may be explained by an example.

Strength of standard, 16 grams of hemoglobin per 100 c.c. of blood.

Comparison of cyanhemoglobin in colorimeter, Standard 10, Unknown 12.

Hence, Unknown has $10/12$ of 16 or 13.33 grams of blood pigment per 100 c.c.

Gasometric determination of hemoglobin, 12 grams per 100 c.c. of blood.

Hence, Unknown has $13.33 - 12$ or 1.33 grams of methemoglobin per 100 c.c. of blood.

Carbon-monoxid Hemoglobin.

This pigment is a molecular combination of one molecule of hemoglobin with one molecule of CO. This combination is stronger than those of hemoglobin and oxygen. The oxygen of hemoglobin is easily replaced, therefore, by CO and, in consequence, the tissues suffer for want of oxygen. This pigment imparts to the blood a bright cherry-red color both in the venous and arterial circulation.

The most common cause of formation of the pigment is the inhalation of coal gas or of illuminating gas. The characteristic color in these cases may disappear after a few hours or it may persist for days, depending on the severity of the case, being frequently found in the blood after death. Such blood shows an absorption spectrum similar to that of oxyhemoglobin, but the two bands lie more toward the violet end of the spectrum than in the case of oxyhemoglobin. On the addition of a reducing agent to blood showing this spectrum, the absorption bands of carbon-monoxid hemoglobin are unaffected, while those of oxyhemoglobin are changed to those of hemoglobin. This spectroscopic test is not delicate as less than 20 per cent. of carbon-monoxid hemoglobin in the blood is difficultly, if at all, detected.

Quantitative Determination

Method of Van Slyke and Salvesen.

The principle of this method¹ is to set free the oxygen and carbon monoxid from their combination with hemoglobin in the blood by addition of ferri-cyanid and then to remove both gases with the help of a Torricellian vacuum in the apparatus discussed on page 441. The oxygen is absorbed in the apparatus by alkaline pyrogallate and the volume of residual carbon monoxid is measured directly at atmospheric pressure, a correction being made for the

¹ Jour. Biol. Chem., 1919, XL, 103.

small and constant amount of nitrogen gas physically dissolved by the blood. From the recent work of O'Brien and Parker,¹ who absorbed the carbon monoxid with a solution of ammoniacal cuprous chloride and, thus, determined this gas directly, it is evident that the amount of CO dissolved in the blood serum, used in the test, is so small that there is little to be gained by making a correction for this factor, as is done by Van Slyke in his method for oxygen of the blood and by Stadie for methemoglobin.

The procedure is, up to the time when the expelled gas is measured, exactly the same as that for the oxygen capacity of the blood (discussed on page 478). The same amount of blood is used and the same solutions are employed, and only the shaking has to be continued a little longer before a constant reading is obtained. This takes about 2 to 3 minutes and is a little different for different species of blood. When the reading of the volume of the gas mixture, consisting of oxygen, carbon monoxid, and a little nitrogen is constant, a solution of alkaline pyrogallate (prepared by dissolving 10 grams of pyrogalllic acid in 200 c.c. of strong potassium hydroxid, consisting of 160 grams KOH dissolved in 130 c.c. of water) is introduced into the cup of the apparatus, is covered by a thin layer of paraffin oil, and is allowed to flow slowly down into the inner wall of the graduated part of the apparatus. A little suction is produced during this part of the procedure by lowering the levelling bulb slightly. The absorption of the oxygen is quite rapid and is completed in less than 1 minute; the reading is taken and the pyrogallate solution introduced once more until a constant reading is obtained. The gas is then measured under the conditions of prevailing pressure and temperature as described on page 479. As the solution is very dark and it is a little difficult to get good readings of the meniscus, a new meniscus is produced by letting water flow down after the pyrogallate solution; the water floats on top of the fluid and one may obtain readings to about 0.002 c.c. The apparatus is washed out twice with dilute ammonia solution after each determination.

Calculation.—The gas measured is reduced to standard conditions by multiplying by the factor as given on page 481. If 2 c.c. of blood have been used, the values of this factor as given in Column 3 of the table on page 480 may be used, the result then being, expressed in c.c. of CO per 100 c.c. of blood, from which amount the nitrogen correction, 1.2 c.c., is subtracted.

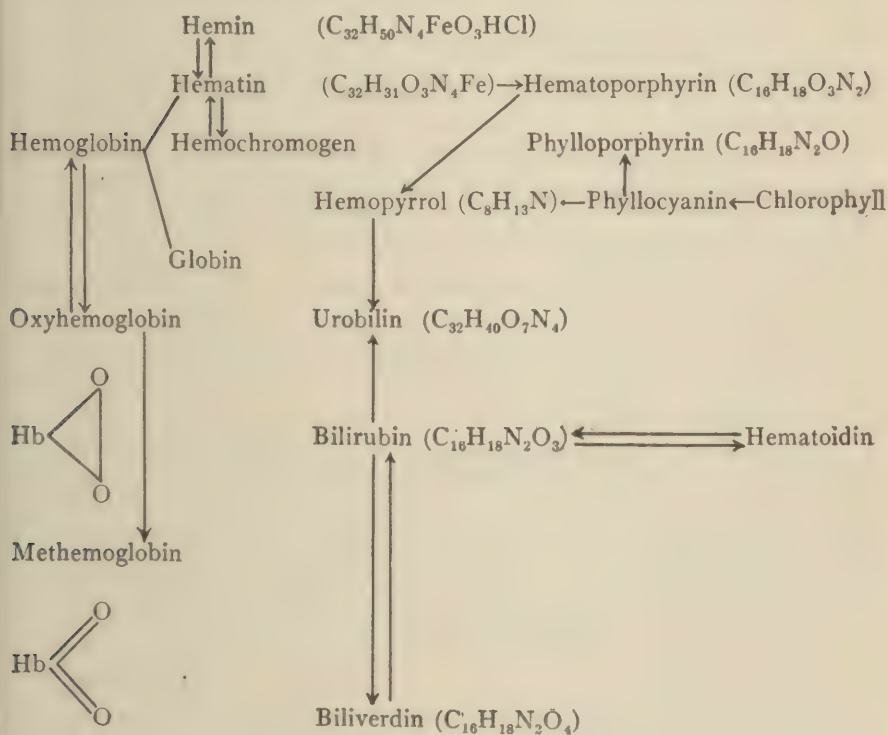
Carbon-dioxid Hemoglobin.

According to Bohr, hemoglobin forms three molecular combinations with CO₂, in which products, α , β , and γ , one gram of hemoglobin combines with 1.5, 3, and 6 c.c. of CO₂. The spectrum of these compounds is similar to that of reduced hemoglobin. If a large excess of CO₂ be present the hemoglobin is decomposed and globin is precipitated. The absorption band under these conditions is probably referable to the presence of free hemochromogen.

¹ *Ibid.*, 1922, L, 289. See also, Nicloux, *Bull. soc. chim. biol.*, 1919, I, 114; *Ibid.*, 1920, II, 171; Hartridge, *Jour. Physiol.*, 1920, LIII, LXXVII; Hill, *Biochem. Jour.*, 1921, XV, 577; Sayers and Yant, *Jour. Am. Med. Assoc.*, 1922, LXXVIII, 1745.

Sulph-hemoglobin.

This pigment, formed by the action of hydrogen sulphid upon hemoglobin, is found in the corpuscles and not free in the plasma. Sulph-hemoglobinemia has been reported by several, especially by van der Bergh¹ whose name is given to the condition known as "idopathic enterogenous cyanosis" and characterized by cyanosis, headache and marked constipation. Clarke² has reported the first case in the United States. Spectroscopic examination of the diluted blood is essential to show the presence of this pigment. The spectrum shows 3 absorption bands, two similar to those of oxyhemoglobin and a third in the red not so near the C line as is the third band of methemoglobin. Addition of dilute ammonium sulphid destroys the third methemoglobin band, while it does not effect that due to sulph-hemoglobin.



Decomposition Products of Blood Pigments.

As stated previously, hemoglobin and oxyhemoglobin are proteins, which are converted under the action of different physical and chemical means into a globulin-like protein, globin, and an iron-containing pigment, hemochromogen. From the table above, adapted from Webster and Koch,³ may be seen

¹ Deutsch. Arch. f. klin. Med., 1905, LXXXIII, 86; Berl. klin. Wchnschr., 1906, XLIII, 7.

² Medical Record, 1909, LXXVI, 143; 1910, LXXVIII, 987. See, also, Wallis, Quart. Jour. Med., 1913, VII, 73; Jamieson, Ibid., 1919, XII, 81.

³ Laboratory Manual of Physiological Chemistry, Chicago, 1903. See Küster, Ztsch. f. physiol. Chem., 1916, XCV, 152.

the relations of hemoglobin to its various derivatives and also to the bile pigments¹ and to chlorophyll.

Hematin.

This decomposition product is found in any situation in which oxyhemoglobin is destroyed; thus, in the digestive tract where it is formed by the action of gastric and pancreatic juice on oxyhemoglobin, in old extravasations, in the stools after hemorrhage, in urine after poisoning with arsenical compounds, and in the blood of persons poisoned with nitrobenzol and acetanilid.² Hematin is a dark brown or blue-black amorphous powder, insoluble in water, dilute acids, alcohol, ether, and chloroform, but readily soluble in acidified alcohol or ether and in dilute alkalies.

Arnold has shown that there are three modifications of this pigment, a neutral, an alkaline, and an acid hematin, each showing spectroscopic differences. The neutral hematin has little importance clinically and will be passed in this discussion. Acid-hematin solutions show four absorption bands, one between *C* and *D*, a second broad but not clearly defined band between *D* and *F*, which divides under certain conditions into two narrower bands, a fourth band between *D* and *E* which is nearer *D* and is very weak. Usually only the band between *C* and *D* and the broad band between *D* and *F* can be seen (see plate).³

Solutions of alkaline hematin show one absorption band between *C* and *D* which reaches out to some extent between *D* and *E*. If the alkaline hematin solution be reduced with ammonium sulphid, the spectrum of hemochromogen is observed, which shows two characteristic absorption bands, one very sharp and dark between *D* and *E* and a second paler and broader band covering the *E* line.

Hematin forms very characteristic compounds with hydrochloric, hydrobromic, or hydriodic acids. With *HCl* hematin crystallizes with one molecule to form the compound hemin. The crystals are light or dark brown rhombic forms and are called, after their discoverer, Teichmann's crystals. Their formation is specific for blood, but the kind of blood cannot be determined by their presence. As their detection is largely a medicolegal question, I will refer a discussion of the technic to the later section.

Hematoporphyrin.

When hematin is treated with concentrated sulphuric acid in presence of air, iron is split off leaving the pigment hematoporphyrin. If air be excluded the product yielded by such treatment is hematin.

This pigment is insoluble in water, but dissolves in alcohol, strong and weak alkalies, and in acids. It is isomeric with bilirubin, with which it is associated in the liver cells. In acid solution hematoporphyrin shows two absorption bands, one fainter and narrower between *C* and *D* and nearer

¹ See Austin and Pepper, *Jour. Exper. Med.*, 1915, XXII, 675; Krumbhaar, Musser and Peet, *Ibid.*, 1916, XXIII, 87 and 97; Hooper and Whipple, *Ibid.*, 137.

² Schumm (*Ztschr. f. physiol. Chem.*, 1912, LXXX, 1) reports hematinemia in a case of acute chromium poisoning; *Ibid.*, 1916, XCVII, 32.

³ See Newcomer, *Jour. Biol. Chem.*, 1919, XXXVII, 465.

D, the other darker, sharper and broader in the middle between *D* and *E*. In dilute alkaline solutions this pigment shows four absorption bands. A band between *C* and *D*, a second broader band surrounding *D* with the broadest part between *D* and *E*, a third between *D* and *E* nearly at *E*, and a fourth broad and dark band between *E* and *F*. Hematoporphyrin is of great importance from the medicolegal standpoint as certain suspected stains may be identified only by its spectrum. For this phase see the later sections.

Hematoidin.

This ruby-red or reddish-yellow pigment is derived from blood coloring matter and like hematoporphyrin is iron free. It is found in old blood-clots, in hemorrhagic exudates, in sputum, and in feces. It is more abundant when the blood pigment is not much exposed to the action of living cells, as in the center of large extravasations and in hemorrhages into preformed cavities of the body (Ziegler). Hematoidin is identical with bilirubin and shows no absorption bands, but only a strong absorption of the violet to the green portion of the spectrum.

Hemosiderin.

This yellow, orange, or brown pigment is a derivative of hemoglobin and contains iron. Unlike hematoidin, it is found more particularly in extravasated blood which has been subjected to the action of living cells. After a time this pigment changes into one (probably hematoidin) which contains no iron.

Malarial Pigment.

The older view of Ewing¹ and others that this yellowish-brown or black pigment was melanin seems to have been disproven by the later work of Brown² who has shown that it is, probably, identical with hematin and is formed by the action of the malarial parasite (possibly of a proteolytic enzyme) upon hemoglobin. On spectroscopic examination of an alkaline alcoholic solution of this pigment, a single broad band is seen starting sharply at *D* and extending to the left, gradually shading into the red between *C* and *D*. The melanins, observed in the leucocytes in relapsing fever, melanotic sarcoma and Addison's disease, are quite distinct from this malarial pigment as shown by decolorizing and solubility reactions.

Estimation of Hemoglobin.

A very large number of methods have been introduced for the determination of the blood coloring matter. Some of these are extremely accurate, but are too complicated for general clinical purposes; others are less accurate, but are more applicable to our work. It may be said that, as a rule, the clinical instruments at our command will give comparative figures, providing their construction and their standardization is accurate. However, we find so much inaccuracy or even lack of proper standardization in some of these instruments, that no absolute comparison may be attempted between the results obtained by various workers using the same or different methods.

¹ Jour. Exper. Med., 1905, VI, 119.

² Arch. Int. Med., 1911, XIII, 290.

Normal adult blood contains about 16.92 grams of hemoglobin per 100 c.c. of blood, this figure being subject, however, to variations at different periods of life, so that these instruments which are standardized against normal adult blood and which furnish the amount of hemoglobin in terms of percentage of such blood cannot be absolutely accurate no matter how perfectly constructed or how accurately standardized, when they are used for the estimation of hemoglobin in the blood of a child or of an elderly patient. It is, therefore, much better practice to obtain the values in terms of the actual amount per 100 c.c. of blood.¹ It is well to recognize, as Türk points out, that we are working with possible errors and can obtain comparative results only when we use the same exactitude and care in each of our estimations. The errors are essentially constant, but we must remember that the arbitrary color standards change as time goes on, necessitating a restandardization of our instruments if accurate results are to follow. The writer has seen errors of 50 per cent. arise from the use of an old von Fleischl instrument and one of 25 per cent. from a faulty standardization of Sahli's solution.

It must be insisted upon that we must not assume that a patient, who is pale and "anemic looking," is in reality anemic. Pallor depends not only upon the amount of pigment in the blood but also upon the delicacy and transparency of the skin and upon the superficial distribution and size of the blood-vessels. By remembering these points we may often save ourselves unnecessary chagrin on finding that the results of our determinations do not accord with the "anemic" expression of the patient. A simple puncture of the ear, allowing the blood to drop upon a clean linen towel (so-called "towel-test"), will many times set us right and prevent a diagnosis of anemia without further examination of the blood. Such examinationless diagnoses are unwarranted and inexcusable.

Direct Methods of Estimation.

These methods are, owing to their complexity, not applicable to clinical work. The spectrophotometer of Hüfner is undoubtedly the very best and most accurate method, but I must refer to the original article for its description. Likewise the colorimetric double pipet of Hoppe-Seyler and the methods of Nebelthau and of Zangemeister must be neglected.

Method of Van Slyke.

This method² is similar to the one for the CO₂ combining power of the

¹ Williamson (Arch. Int. Med., 1916, XVIII, 505) has emphasized this point. His examination of 157 cases between the ages of 16 and 60 years shows that the hemoglobin, in the case of males, is 16.92 grams per 100 c.c., while with females it is 15.53. In 227 cases between the ages of 1 day and 16 years shows, males, 16.20 grams; females, 16.38 grams.

² Jour. Biol. Chem., 1918, XXXIII, 127; Lundsgaard and Möller, Ibid., 1922, LII, 377. For other methods and points in connection with the chemistry of hemoglobin see Palmer, Jour. Biol. Chem., 1918, XXXIII, 119; Berman, Ibid., XXXV, 231; Arch. Int. Med., 1919, XXIV, 553; Cohen and Smith, Jour. Biol. Chem., 1919, XXXIX, 489; Newcomber, Ibid., XXXVII, 465; Herzfeld and Klinger, Biochem. Ztschr., 1919, C, 64; Wertheimer, Ibid., 1920, CVI, 12; Smith, Dawson, and Cohen, Proc. Soc. Exp. Biol. & Med., 1920, XVII, 211; Welker and Williamson, Jour. Biol. Chem., 1920, XLI, 75; Howard, Ibid., 537; Robscheit; Ibid., XLII, 209; Adolph and Ferry, Ibid., 1921, XLVII, 547; Terrill, Ibid., 1922, LIII, 179; Whipple, Arch. Int. Med., 1922, XXIX, 711. Hektoen and Schulhof (Jour. Inf. Dis., 1922, XXXI, 32) announce the production of specific erythroprecipitins (hemoglobin precipitins).

blood previously discussed and consists in the measurement of the oxygen set free from the hemoglobin of laked blood. From the volume of this oxygen, the oxygen bound by the hemoglobin may be calculated and, by a simple estimation the actual amount of hemoglobin present determined. For scientific determinations this method is to be recommended, although it will hardly supplant the indirect methods of hemoglobin estimation for clinical work.

Three or more c.c. of blood are introduced into a separatory funnel or bottle and distributed in a thin layer about the inner wall, so that maximum contact with the air and complete saturation of the hemoglobin with oxygen are assured. The vessel is rotated for a few minutes so that the blood is kept in a thin layer, or it may be shaken for 15 minutes or longer on a mechanical shaker. The saturated blood is then transferred to a cylinder or a heavy walled test-tube.

The blood gas apparatus previously described (page 441) is now prepared by introducing into it 5 drops of redistilled caprylic alcohol and 6 c.c. of ammonia solution made by diluting 4 c.c. of concentrated ammonia to a liter. If saponin powder is available, as much is added to the 6 c.c. of ammonia, while in the cup of the apparatus, as will stick to the end of a glass rod (approximately 1 mg. per c.c.). After the ammonia is introduced into the 50 c.c. chamber of the apparatus, the latter is evacuated in the manner previously described and the air is extracted from the ammonia solution by shaking for about 15 seconds. The extracted air is expelled, and the extraction repeated to make sure that no air is left in the solution. Finally, about 2 c.c. of the air-free ammonia are forced up into the cup of the apparatus. The aerated blood is now thoroughly stirred with a rod to assure even distribution of the corpuscles, and a sample is drawn into a 2 c.c. pipet and run under the ammonia in the cup of the apparatus. (The lower delivery mark of the pipet should be 3 or 4 cm. above the tip as a pipet calibrated for complete delivery would be inconvenient for placing the entire sample of blood below the layer of ammonia.) The blood is now run from the cup into the 50 c.c. chamber, the ammonia layer following the blood and washing it in. A few additional drops of the air-free ammonia may if necessary be added from a dropper to make the washing complete.

The blood and ammonia in the chamber are mixed and allowed to stand until the blood is *completely laked*. This requires about 30 seconds when saponin is present and 5 minutes when it is not. After laking is complete, 0.4 c.c. of a saturated potassium ferricyanide solution is introduced to set free the oxygen combined with the hemoglobin. (This ferricyanide solution is made by dissolving 40 grams of the salt in 100 c.c. of water and is made air-free by boiling or shaking in an evacuated flask and is kept in a buret under a layer of paraffin oil 2 or 3 cm. thick to exclude air.) The apparatus is now evacuated by lowering the levelling bulb, until only a few drops of mercury remain above the lower stop-cock, and is shaken, preferably with a rotary motion, to whirl the blood in a thin layer around the wall of the chamber. If the blood was completely laked before the cyanid was added, extraction

of the oxygen may be completed by $\frac{1}{2}$ minute of efficient shaking. The extracted solution may be drawn into the bulb of the apparatus below the lower cock and the extracted gas measured over mercury as in the determination of CO_2 previously discussed. After the gas volume has been read, the chamber is evacuated and the blood readmitted and shaken again for $\frac{1}{2}$ minute. If the reading shows no increase, it is evidence that all the oxygen was extracted by the first evacuation; if there is an increase, the extraction must be repeated again. After each analysis, it is well to wash out the 50 FACTORS FOR CALCULATING HEMOGLOBIN FROM OXYGEN BOUND BY 2 c.c. OF BLOOD

Temperature	Air dissolved by 2 c.c. of blood. Subtract from gas volume read in apparatus in order to obtain <i>corrected gas volume</i> , representing O_2 , set free from hemoglobin.	Factor by which corrected gas volume is multiplied in order to give:	
		Oxygen chemically bound by 100 c.c. of blood.	Per cent hemoglobin calculated on the basis: 18.5 per cent. oxygen = 100 per cent. hemoglobin.
C°.	c.c.	c.c.	Per Cent.
15	0.037	$46.5 \times \frac{B}{760}$	$251 \times \frac{B}{760}$
16	0.036	$46.3 \times \frac{B}{760}$	$250 \times \frac{B}{760}$
17	0.036	$46.0 \times \frac{B}{760}$	$249 \times \frac{B}{760}$
18	0.035	$45.8 \times \frac{B}{760}$	$247 \times \frac{B}{760}$
19	0.035	$45.6 \times \frac{B}{760}$	$246 \times \frac{B}{760}$
20	0.034	$45.4 \times \frac{B}{760}$	$245 \times \frac{B}{760}$
21	0.033	$45.1 \times \frac{B}{760}$	$244 \times \frac{B}{760}$
22	0.033	$44.0 \times \frac{B}{760}$	$242 \times \frac{B}{760}$
23	0.032	$44.7 \times \frac{B}{760}$	$241 \times \frac{B}{760}$
24	0.032	$44.4 \times \frac{B}{760}$	$240 \times \frac{B}{760}$
25	0.031	$44.2 \times \frac{B}{760}$	$239 \times \frac{B}{760}$
26	0.030	$44.0 \times \frac{B}{760}$	$237 \times \frac{B}{760}$
27	0.030	$43.7 \times \frac{B}{760}$	$236 \times \frac{B}{760}$
28	0.029	$43.5 \times \frac{B}{760}$	$235 \times \frac{B}{760}$
29	0.029	$43.3 \times \frac{B}{760}$	$234 \times \frac{B}{760}$
30	0.028	$43.1 \times \frac{B}{760}$	$233 \times \frac{B}{760}$

c.c. chamber of the apparatus with the dilute ammonia solution, as a black precipitate is formed by reaction of the reagents with the mercury.

In order to calculate the oxygen bound by the hemoglobin, it is necessary to subtract, from the gas measured, the volume of air physically dissolved by the 2 c.c. of blood at atmospheric pressure and the prevailing room temperature. The volume of gas thus corrected may be reduced to standard conditions of 0° and 760 mm. by multiplying by $(0.999 - 0.0046t) \times \frac{\text{barometer}}{760}$, t being the temperature Centigrade at which the test was made. If this result be multiplied by 50, the figure obtained will represent the cubic centimeters of oxygen bound by the hemoglobin in 100 c.c. of blood. The volumes of air dissolved by the blood at different temperatures are given in the table above. This table also gives factors by which one may transpose the readings directly into terms of volume per cent. of chemically bound oxygen in the blood, or of per cent. hemoglobin on the basis of Haldane's normal average, 18.5 per cent. of oxygen in the blood being taken as equivalent to 100 per cent. hemoglobin.

It is advisable, after one 2 c.c. portion of a blood sample has been analyzed to aerate the remainder a second time and repeat the determination, in order to make certain that the hemoglobin of the first portion was completely saturated with oxygen, this method giving a check on the results.

The following example illustrates the calculation:

Observed gas volume, at 20° and 750 mm.....	0.450
Correction for dissolved air.....	0.034
Corrected gas volume.....	0.416
$0.416 \times 44.8 =$	18.65 volume per cent. oxygen.
$0.416 \times 243 =$	101 per cent. hemoglobin.

Indirect Colorimetric Methods.

These indirect methods employ a comparison of the blood solution with a second medium which approximates the blood in color. Two principles are possible in such methods. We may either have a fixed unchangeable medium of comparison, with which the blood is matched by constant dilution, or we may use a fixed blood solution of known strength and compare therewith the standard graduated color scale. Both of these principles have been used in such determinations, the variations being shown in the fact that instruments for this purpose have been introduced by Gowers, Sahli, Hayem, Malassez, Henocque, Bizzozero, von Fleischl, Miescher, Haldane, Grützner, Gärtner, Dare, Oliver, and Tallqvist.

It is evident, *a priori*, that these indirect methods must have greater errors than the direct. Two things are essential for any exactitude whatever. First, the medium of comparison must agree in the most complete manner with the various color tones which the blood shows at different percentage values of hemoglobin. Second, the standardization of the medium of comparison must be extremely accurate. We must have no illusions regarding the exactness of the hemoglobin values obtained by these methods, as it is very difficult to construct two instruments of the same kind that will agree exactly. Certainly no two instruments of different make will agree, but comparative results sufficiently accurate for clinical purposes are obtained by

the use of the more reliable instruments. The personal equation in reading the color comparisons must be remembered, as some individuals show abnormal sensitiveness or lack of sensitiveness to shadings of red. It is an impossibility in this book to give in detail all of the methods advanced for the estimation of hemoglobin. I select, therefore, those that have proven most reliable in my hands.

Hemometer of Fleischl-Miescher.

Up to recent times the most frequently used of the instruments for the estimation of hemoglobin was the old von Fleischl instrument. With the introduction of the Miescher modification, this original form has been or should be less often employed. We avoid, therefore, a discussion of the older instrument, referring to other works which have included it.

This new apparatus, made by Reichert, under the direction of Miescher,

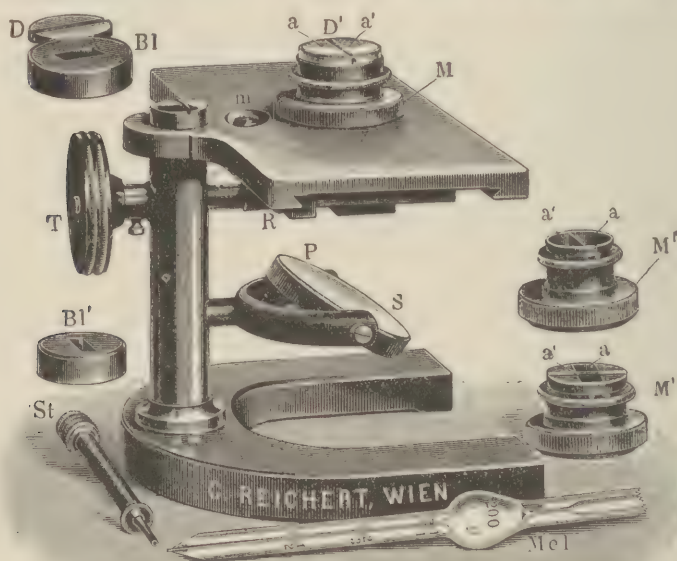


FIG. 125.—Hemometer of Fleischl-Miescher: *R*, Stage; *T*, milled head, which moves the color scale; *m*, opening in stage through which the instrument is read; *M*, mixing cell; *D'*, cover glass; *D*, cap; *PS*, gypsum mirror from which light is reflected; *mel*, diluting pipet.

is similar in general appearance to the old von Fleischl. It has the same stand and the same scale principle, although this latter is standardized differently and graduated on a different basis. It differs, materially, in the method of measuring and diluting the blood, in the form of the comparison chamber, and in the meaning of the graduation of the scale (see cut and legend for its description).

The Diluting Pipet.

This is similar in construction to the pipet of the Thoma-Zeiss hemocytometer, its calibrations, however, being different. The marks are $\frac{1}{2}$, $\frac{2}{3}$, and 1. Above and below each of these main divisions are two marks each corresponding to $\frac{1}{100}$ of the contents of the capillary tube. This device

enables the worker to measure accurately the column of blood taken, in case he gets too little or too much blood in the tube. The relation of the capillary to the ampulla is such that blood, drawn to the mark 1 and diluted to the mark above the ampulla, receives a dilution of 200; if drawn to the mark $\frac{2}{3}$ the dilution is 300; while the line $\frac{1}{2}$ furnishes a dilution of 400. The diluent used is $\frac{1}{10}$ per cent. sodium carbonate solution. This dissolves the stromata of the red cells furnishing a clear solution. Occasionally the diluent becomes turbid after standing some time, and should be freshly made and should contain no bicarbonate.

The taking of the blood and the mixing with a diluent is done with the same precautions mentioned under the method of making the blood count. In choosing the proper dilution, one should select that which will enable him to use the central portions of the graduated scale. A dilution of 400 is usually applicable except in cases in which marked anemia is suspected when dilutions of 300, 200, or even 100 should be made. In making this last dilution the erythrocytometer may be used. In doing accurate work, it is well to make a preliminary determination of hemoglobin in order to tell, the better, just what dilution would be advisable. This may be done by the Tallqvist method described later.

The Comparison Cells.

With this instrument two cells for holding the blood and diluent are furnished. One of these has a depth of 15 mm., the other one of 12 mm. The former is the standard cell, the latter the control, giving $\frac{4}{5}$ the value of the larger cell. Their external appearance is similar to the cell of the old Fleischl, but their capacity is less owing to their greater thickness.

The dividing partition between the halves of the cells projects about $\frac{1}{2}$ mm. above the borders, thus preventing any mixing of the fluids in the two portions. A grooved cover-glass is slid over the compartments without fear of mixing the fluids. If fluid is lost no error is introduced, as the dilution is uniform in the pipet and the depth of the chamber is definite. A diaphragm, with an opening 4 mm. wide, is placed over the cover-glass after this latter has been adjusted, the opening being so placed that its long axis is perpendicular to that of the vertical partition. The field of comparison is thus limited to 4 mm., which corresponds to about 3° of the scale, affording a comparison of a single tint of the scale with the color of the diluted blood.

Graduation of the Scale.

The slide for comparison of color is tinted with Cassius' golden-purple, as in the old Fleischl instrument. The graduations of this color scale are made by comparison with standard solutions of hemoglobin and not with the arbitrary standard of blood of so-called normal individuals. The scale shows the same divisions as that of the Fleischl instrument, but their significance is different in the Miescher modification. Here one does not read directly the percentage of hemoglobin, but must obtain the corresponding value by reference to a "table of calibrations," which accompanies each instrument. While the figure thus obtained is in terms of actual percentage (grams per 100 c.c.), it is quite different from the percentage figure given by the Fleischl instrument.

Method.

After testing the chamber by placing in one compartment the diluting fluid in order to see that none runs into the other, the blood is drawn into the pipet to the desired point and diluted to the mark, all precautions mentioned under Blood Counting being observed. Thoroughly mix the blood and diluent by shaking and blow out the unmixed contents of the capillary tube. Fill one compartment of the 15 mm. cell with the diluted blood so that a convex meniscus appears above the border of the chamber, the other compartment being filled with the diluent or with distilled water. Adjust the grooved slide and cap, place the cell in the central opening of the stand, and adjust the light. This latter portion of the technic is of considerable moment, as this instrument gives the best results when used in a dark room illuminated only by a small candle flame which is placed about 18 inches from the stand and to the side. In the absence of a dark room, a light-proof shield for the eyes may serve. This may readily be obtained by the use of a tube of stiff, dark paper, which fits over the comparison cell and shuts off the light of the candle from the field of vision. The observer should stand in such a position that he looks into the cell from the side and not from the front or back, the eyes being about one foot above the cell. In the comparison of the color tones the variations are much better seen by quick movements of the slide rather than by slow gradual changes. The eyes should be rested at short intervals to prevent fatigue and lack of sensitiveness to the different shadings of color. When the color is matched take the reading of the instrument, by observing what mark of the scale coincides with the notch on the edge of the opening above the scale, and control this reading by several duplicates.

Remove the blood from the 15 mm. cell by means of the pipet and transfer it to one compartment of the 12 mm. chamber, tested as was the larger one. Adjust this chamber and make readings in the same way. These values should be only four-fifths of those obtained with the 15 mm. cell. This modification has the advantage of using different portions of the color scale and should give comparative figures. If there should be any variation, which should never be over 1 per cent., a correction may be made.

Calculation of Results.

This is possible only with the use of the "table of calibrations," which contains the series of scale divisions and the absolute amount of hemoglobin in mg. per 1,000 c.c. of blood, corresponding to each division of the scale when the 15 mm. chamber is used. Thus, if the scale shows 56, we find the value corresponding to this to be 447 mg. of hemoglobin in 1,000 c.c. of diluted blood. As the dilution may have been 400, we would have, in 1,000 c.c. of undiluted blood, $400 \times 447 = 178,800$ grams. As we wish to know the amount in 100 c.c. we have merely to divide by 10, thus obtaining 17,880 grams of hemoglobin. If we wish to get the percentage figures, as read on the old Fleischl, we divide the figure obtained with the Miescher, in this case 17.88, by the amount of hemoglobin corresponding to the 100 division of the scale of the instrument used, in this case 14, and we obtain 127.7 per cent.

This Miescher modification is our very best clinical apparatus for estimation of hemoglobin, giving results which are accurate within 0.2 to 0.5 per

cent. It is open to the objections that it is bulky, expensive, and requires more time and practice for its use than is at the control of the busy practitioner. For hospital use, however, it is the instrument *par excellence* and should never be substituted by others. It has one disadvantage, that each instrument is standardized, not against a known hemoglobin solution, but against a so-called "normal" instrument, which has been properly standardized. It is to be hoped that each instrument will, in the future, be properly calibrated and thus insure us against the possibility of errors arising from any change in the standardization of the "normal" instrument.

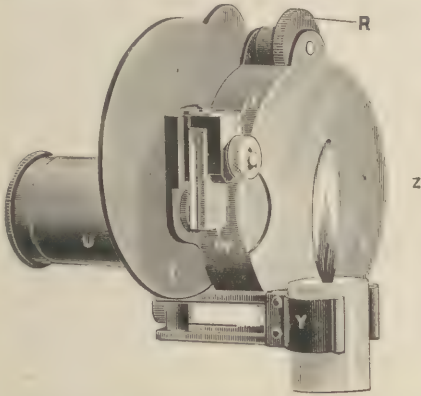


FIG. 126.—Hemoglobinometer of Dare: R, milled wheel; S, case inclosing the color disk; T, movable wing, which is swung outward; V, telescoping camera; v, aperture admitting light; w, capillary blood pipet; x, detachable candle holder; z, slot through which the percentage of hemoglobin is read.

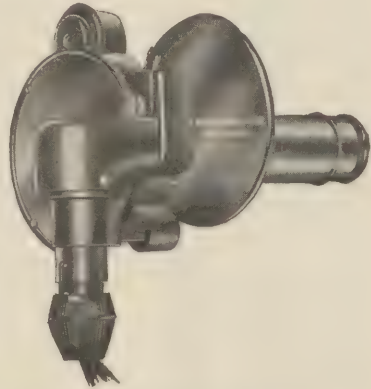


FIG. 127.—Dare's hemoglobinometer with electric attachment.

Hemoglobinometer of Dare.

The instrument, introduced by Dare, has the advantage of using undiluted blood, and avoids any error consequent upon dilution. The principle of this instrument is as follows: The color of undiluted blood is compared by artificial light with that of a graduated glass scale colored with golden-purple, the 100-point of which is standardized against a solution of 13.77 grams of hemoglobin in 100 c.c. of serum. For a description of the apparatus see cut.

Method.

Swing outward the movable screen, which serves as a cover for the case, adjust the camera tube, and fit the candle attachment in its place opposite the camera tube. The candle should be so adjusted that its upper end is flush with the top of the clips which hold it. If the wick be curved, it should be so turned that the intensity of the light is midway between the two apertures. See that the pipet, composed of the rectangular glass plates, is thoroughly cleansed and dry. The space between the plates is filled by applying the edge of the pipet to the side of a fairly large drop of blood. Adjust this pipet in its place and rotate the colored scale, by means of the milled screw, until the colors match. Hold the instrument steady to prevent the flickering of the flame as much as possible. No dark room is necessary,

but it is advisable to point the instrument at some dark object and to avoid direct sunlight, as the shadings of color are not so easily matched by direct daylight. As soon as the colors are matched make the readings and check the results with several duplicates. This reading is observed on the left side of the case in the small open space, the line which coincides with the beveled edge of the opening representing the percentage of hemoglobin, on the basis of a value of 13.77 grams of hemoglobin per 100 c.c. as 100 per cent. It is, therefore, easy to calculate the direct amount of hemoglobin in the blood examined.

This instrument has the advantages that undiluted blood is used, that the scale of comparison is usually very accurately standardized, that it is convenient, easy of manipulation, and rapid in giving results. Coagulation of the blood does not occur sufficiently soon to introduce an error, providing the reading is taken within a reasonable time. It is more convenient for general

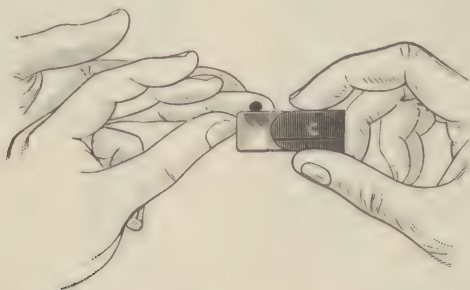


FIG. 128.—Method of filling the Dare blood pipet. (*Da Costa.*)

use than is the Miescher, is less expensive, can be used in a light room, and gives results second only to those of the Miescher. The disadvantages of this instrument are that an occasional faulty standardization may introduce errors, it costs much more than some of the instruments to be described, and it is not a long-lived instrument unless care is taken in handling it. In the writer's laboratory this instrument has given great satisfaction and can be recommended for general use on the ground of its convenience and ready application to clinical work.

Hemometer of Sahli.

This instrument is a new modification of the older hemoglobinometer of Gowers, and has so many advantages over the older instrument that this latter will be passed over. A modification of the Gowers instrument introduced by Haldane is simple and reliable, but has the disadvantage that coal gas is used in converting the hemoglobin into CO hemoglobin, and consequently, is not of easy application for bedside work.

As Sahli has pointed out, a colored fluid under examination should not be compared with a different substance similar in color, but with a solution of known strength of the same coloring matter. His modification of the Gowers method employs an instrument constructed on exactly the same lines, but using a different standard of comparison. This standard of comparison is an acid hematin solution¹ in a concentration corresponding to a 1 per cent.

¹ Jacobson (*Jour. A. M. A.*, 1919, LXXIII, 1282) proposes the use as a standard in this test, of a solution of gallic or tannic acid treated with concentrated sulphuric acid forming

solution of normal blood. This solution is somewhat dark, as it is standardized against blood showing high hemoglobin value. As Sahli states, the standard fluid, as furnished with his hemometer, corresponds to a blood which shows with the Miescher instrument at a dilution of 200 a reading of 109, or an absolute quantity of 17.2 grams of hemoglobin per 100 c.c. of blood. The fine particles of hemin, which are in suspension, may adhere to the glass, especially if the instrument lies unused for some time. This process changes the color of the standard to some extent, so that it is supposed that deterioration has occurred. This may be remedied by completely inverting the tube, without violent shaking, thus allowing the precipitate to diffuse uniformly. Occasionally one of these instruments is found to be improperly standardized, but this is rare.

Method.

This consists in diluting the blood with 10 times its volume of N/10 normal hydrochloric acid. After a few seconds the fluid becomes dark brown from the formation of hematin hydrochlorid (hemin), which substance is not in solution, but in fine suspension. The blood is taken with the 20 mm. pipet and blown into the graduated tube, which contains N/10 normal HCl up to the mark 10. This acid may be accurately enough made by diluting 15 c.c. of concentrated chemically pure HCl to 1,000 c.c. Shake the mixture of blood and acid and dilute with ordinary water as soon as a clear dark brown color is visible. Add water until the shade of the mixture corresponds exactly with that of the standard solution, when the percentage of hemoglobin may be read off. The water should be added very carefully from a dropping pipet, as the accuracy of the method depends upon adding the exact amount of fluid necessary. The comparison of colors may be made in any light, as the two solutions, being the same, will be similarly affected.

This instrument is very conveniently gotten up, being mounted in a case, carrying a white-glass plate to reflect the light to better advantage. It is simple, inexpensive, and is accurate within 2 per cent. The author would recommend this instrument to the general worker above all others, with the possible exception of the Dare, whose advantages have been mentioned.

Hemoglobinometer of Oliver.

In this method the color of the blood in a definite dilution is compared, "rufgallic acid." See, also, Kuttner, *Ibid.*, 1916, LXVI, 1370; Haessler and Newcomer, *Arch. Int. Med.*, 1916, XVII, 806; Palmer (*Jour. Biol. Chem.*, 1918, XXXIII, 119) advises the use of CO-hemoglobin as a standard. See, also, Cohen, Barnett and Smith, *Ibid.*, 1910, XXXIX, 489; Berman (*Arch. Int. Med.*, 1919, XXIV, 553) proposes an acid hematin method, modifying the Sahli procedure.

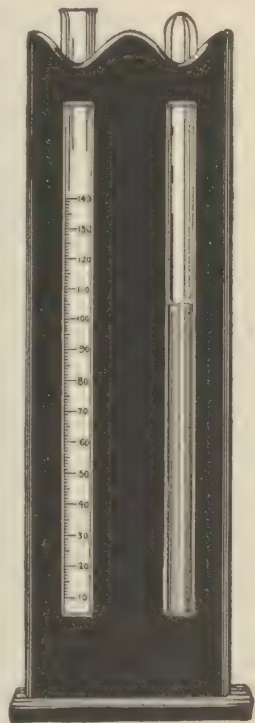


FIG. 129.—Hemometer of Sahli. (Greene.)

by light reflected from a white surface, with a series of tinted glass standards. Such a method has the advantage that the color of the diluted blood is compared with one single tint at one time. The standard glass disks correspond accurately, as determined by the tintometer, to the specific color curve of progressive dilutions of normal blood. Two sets of standards are furnished,

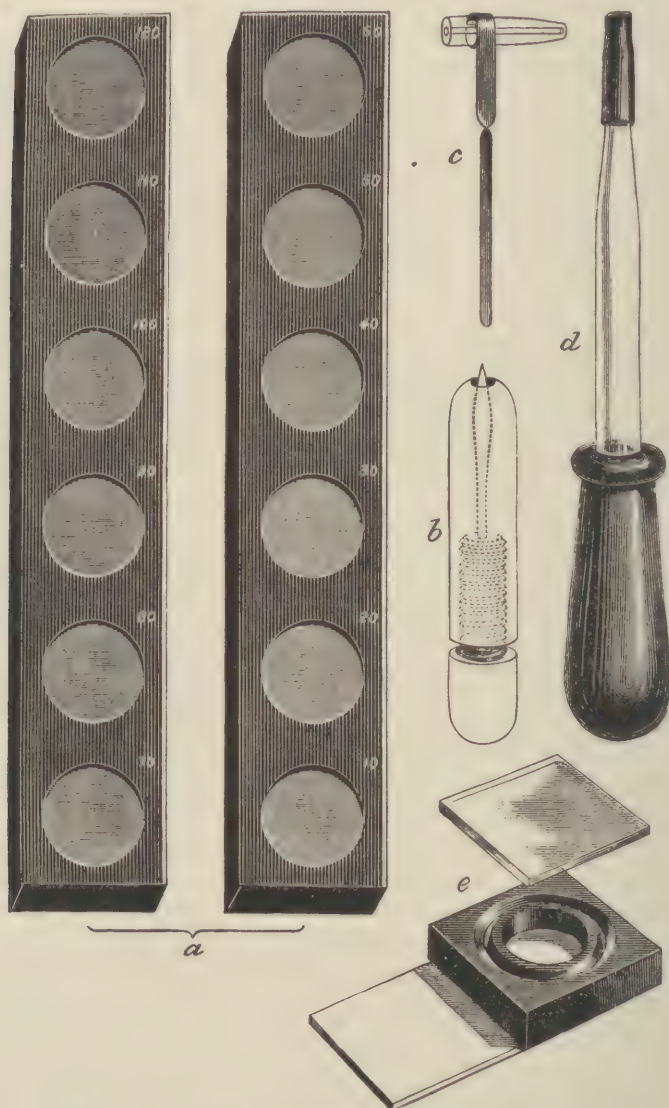


FIG. 130.—Hemoglobinometer of Oliver. (Coplin.)

one for use in daylight, the other by candlelight, the latter being preferable. Each set of 12 disks is mounted on plaster of Paris and enclosed in two wooden frames, six disks in each frame. They represent the color of solutions of hemoglobin with percentages from 10 to 120. To obtain intermediate values, colored riders are used, each representing 2.5 and 5 per cent.

of hemoglobin, when used with the disks from 70 to 120, but twice these values if used with the disks from 10 to 60.

The blood is taken in a capillary pipet similar to that of von Fleischl, holding 5 cmm. of blood. The diluting chamber has a white background of plaster of Paris and, when filled with distilled water in which one pipetful of blood has been dissolved, yields a solution of 1 per cent. When filled the cell is covered with a blue-glass cover.

Method.

The pipet is filled with blood drawn by capillarity from a puncture of the ear or finger. Wash the blood from the pipet into the diluting chamber by means of water from a medicine dropper, fill the chamber with water, stir well with the handle of the pipet, and adjust the cover-glass. A small bubble of air should be included as an evidence that the chamber was not overfilled.

Compare the color of this solution with that of the standard disks, using the light of a candle placed 3 to 4 inches away in such a manner that the light strikes the two chambers alike. It is well to use the camera tube to shield the eyes and enable one to compare the tints more accurately. If the color matches that of any of the disks, the percentage is read directly from the case. If, however, the color is intermediate between two disks, the riders must be superimposed on the disk of lower percentage, and a second comparison made by using a corresponding plate of unstained glass as a rider on the diluting chamber. The adjustment of tints and riders must be continued until the colors match exactly. Naturally the variation will equal 2 per cent. as the ordinary riders are equivalent to $2\frac{1}{2}$ per cent. of hemoglobin. It is not necessary for this determination that the room be absolutely dark.

This method is accurate within the limit mentioned (2.5 to 5 per cent.), but it is trying, time consuming, and does not equal in accuracy the methods of Dare and of Sahli. The instrument is inexpensive and the disks are prone to deteriorate.

Tallqvist's Hemoglobinometer.

Tallqvist has introduced a method for the estimation of hemoglobin which is so simple that certain writers have been led to recommend it above other methods. The principle of this method is essentially the same as Oliver's method, although the application is entirely different. Tallqvist compares the color of the undiluted blood with that of a series of lithographed standard tints, which range by differences of 10° from 10 to 100 per cent. These stand-

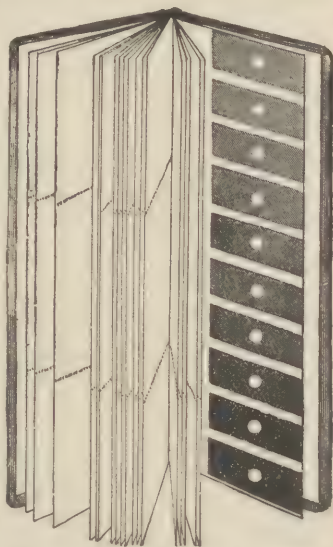


FIG. 131.—Tallqvist's hemoglobinometer.

ard tints were prepared by matching in water colors the tint of the blood of various patients (whose hemoglobin values had been determined with the von Fleischl instrument) when soaked into standard filter-paper. These tints were then lithographed and bound up with sheets of filter-paper, the combination making a very simple and convenient book which may be easily carried in the pocket.

Method.

Allow a fairly good-sized drop of blood to soak into a portion of the filter-paper by holding the edge of the sheet against the drop. Care should be taken to allow this to take place very slowly so that the color may be uniform throughout. If carefully done it will not be necessary to blot the stain, but sometimes this is essential. As soon as the stain has lost its humid gloss compare its color with that of the lithographed scale. Do not allow the stain to become dry, as the color comparisons are disturbed by the coagulation. Hold the scale and the stained paper in such a way that the light (daylight only) is well reflected from the color scale. The percentage of hemoglobin is then read off directly by noticing the point of the scale with which the blood stain exactly corresponds. As this scale does not read closer than 10 per cent., the intermediate percentages must be judged by difference. Here personal factors are of great importance, some workers being so skilled as to detect small variations.

This method can furnish, at best, only an approximate result and has nothing in common with the other methods discussed. It is to be advised only when some more reliable method is not at hand or when a rough estimate only is wanted. It is recommended by some writers as being as generally useful and accurate as any of the other methods, but the writer can see no advantage whatever in its use, as the results obtainable are, in his opinion, not reliable and are not as satisfactory to one who is not especially accustomed to such color comparisons. This test would better be limited to rough, preliminary, approximate determinations than to be used in the more careful estimations which clinical work requires.

In the selection of a method for estimating the hemoglobin of the blood, the writer would advise the Fleischl-Miescher instrument for those who are in close touch with hospital or clinical laboratory facilities. For the general practitioner who must make his own determinations under varied circumstances, the Dare or the Sahli instrument may be unequivocally recommended, the latter having the advantage of cheapness along with accuracy and ease of manipulation.

Variations in the Amount of Hemoglobin.

The percentage values are misleading, as the hemoglobin varies with age and, to some extent, with sex.¹ For this reason it is preferable to obtain the absolute amount of hemoglobin, which is done directly by the Miescher and, by a very simple calculation, with the Dare or Sahli instruments. In those methods which read in direct percentages one may readily calculate

¹ See Williamson, *Jour. Am. Med. Assn.*, 1915, LXV, 302.

the absolute values per 100 c.c. of blood by multiplying the percentages obtained by 0.1377. In estimating the true figures for the hemoglobin in the blood of women, it is necessary to add one-eighth to the percentage values as read, as female blood runs from $12\frac{1}{2}$ to 20 per cent. less in hemoglobin than does the blood of man; likewise for a child we should add one-seventh to the percentage. In this way we correct the readings of the instrument which is calibrated against adult male blood. It has been found by comparative tests, that the blood of rural residents reaches the normal standards much more frequently than does that of their city brothers, this being due, no doubt, to the purer fresh air which the country dweller enjoys.

A relative diminution in the amount of hemoglobin, as determined by the above methods, is known as oligochromemia or as achroicythemia. This condition is usually associated with a decrease in the number of erythrocytes (oligocythemia), but in cases of chlorosis the diminution of hemoglobin is an absolute reduction, each cell showing less hemoglobin than normally and no oligocythemia being, as a rule, present. In pernicious anemia, on the other hand, each cell may show an absolute increase in hemoglobin, although the percentage value is reduced owing to the marked oligocythemia present at the same time. Factors bringing about conditions of hydremia lead to a transient oligochromemia, while factors acting as etiologic units in the production of anhydremia lead to a reverse condition of polychromemia.

Oligochromemia is observed in chlorosis, pernicious anemia, leukemia, and secondary anemias, following chronic infections, hemorrhage, malignant growths, and some constitutional diseases. It is noted in chronic nephritis, chronic enteritis, and mineral poisonings, especially those with lead and mercury compounds. It has been stated that low hemoglobin values sometimes observed in cases which are to have surgical intervention are contraindications to operative treatment as the anesthetics themselves may bring on a condition of oligochromemia. While it is wise to watch with extreme care cases showing less than 50 per cent. hemoglobin, yet surgical operations have been successfully performed on cases with a more marked diminution of the hemoglobin.

Color index.

This term is used to indicate the amount of hemoglobin contained in each cell, as compared with the amount present when a normal number of erythrocytes obtains. In other words, it is the quotient of the hemoglobin percentage divided by the percentage of red cells. This latter factor is obtained by dividing the number of red cells, as found by the count, by the number reckoned as normal, namely, 5,000,000. A very simple method of getting this percentage is to multiply the number of hundreds of thousands of red cells by two; thus if 2,650,000 reds were counted we obtain 53 as the percentage of red cells. Sahli recommends the use of the term "hemoglobin quotient" or "hemoglobin value" for this factor, but the expression color-index has become so general that it will be hard to replace. Moreover, the latter term seems to convey a more definite idea to most of us than would the substitutes suggested.

The color-index is normally one; that is, a hemoglobin value of 100 per

cent. is associated with a blood count of 5,000,000 red cells. We find, however, in the different anemias that this figure varies markedly. In those anemias, in which the reds are diminished to the same relative extent as is the hemoglobin, the index remains normal; while in those cases in which the hemoglobin is markedly reduced without a coincident decrease in the reds, the value is necessarily less than one. This latter condition is observed especially in chlorosis and in splenic anemia, while in the pernicious types of anemia we find the diminution of the reds much greater, proportionally than that of the hemoglobin, a high color index consequently obtaining. In such cases the index may run from 1.02 to 1.9 as in one case observed by the writer.

Such variations are the rule, but are not invariable. We may find the various anemias showing, at times, very unusual color indices. As this factor is intended to show only the relations of the hemoglobin to the cells it must not be taken as absolutely diagnostic, but rather as merely significant. It must be remembered, moreover, that this figure cannot be absolutely exact as it is based on a purely arbitrary number of red cells as the normal value and as the instruments for estimating hemoglobin are often improperly standardized. The results are, however, comparable and often yield valuable clinical information, if individual, racial, and seasonal variations in both the amount of hemoglobin and the number of red cells are taken into consideration.¹

(C) Proteins of the Blood.

From the point of view of physiological chemistry a discussion of the various protein bodies found in the blood embraces, necessarily, those of the intracellular fluid as well as those of the cellular elements. From the clinical standpoint, however, the discussion of this field is limited more or less to the proteins of the serum. I can, therefore, do little more than refer to the fact that the red cells contain, besides the hemoglobin which constitutes about 90 per cent. of their organic matter, a nucleoprotein which shows properties resembling those of both the globulins and albumins. The proteins of the white cells are still little differentiated. Miescher found five different forms of protein, showing various solubility and coagulation relations. Besides these the leucocytes contain, as characteristic proteins, nucleins, which are compounds of the phosphoric acid-containing nucleinic acid with simple albumins. The metabolism of the nucleins is an important factor in various clinical conditions, but I must refer elsewhere for such discussions. Little is known regarding the chemical composition of the blood-plates. Löwit affirms that they are composed principally of globulin, while Lilienfeld asserts that their substance belongs to the class of nucleo-albumins.

In the process of coagulation, fibrinogen, a protein of the plasma, is converted into fibrin through the influence of thrombin (fibrin ferment), whose chemical nature is not absolutely settled; it belongs probably to the class of nucleo-proteins, possessing, however, many of the characteristics of the globulins. Blood serum, of both physiologic and pathologic types, contains two protein bodies, serum globulin and serum albumin. A third body, called by Chabrie albumon, has been assumed, but the researches of Drechsel and of

¹ See Meyer and Butterfield, *Arch. Int. Med.*, 1914, XIV, 94.

Brunner have shown that this body is not preformed in the serum, but arises from serum albumin and serum globulin during the process of coagulation.

According to Hammarsten, normal human blood serum contains 7.62 per cent. of total protein, while Schmidt gives this figure as 8.26 per cent. The researches of Reiss, Strauss and Chajes, and more recently of Engel have shown that the refraction coefficient of serum, in health, is between 1.3487 and 1.3517, corresponding to a percentage of 7.74 to 9.13 of protein. The introduction of the refraction coefficient into the study of the serum and plasma has furnished a method of clinically studying hydremia as well as one by which the water content and serum proteins may be easily estimated.¹ These factors are of great importance in the study of the various anemias, as we know that the serum or plasma is of much more importance, from the pathologic standpoint, than are the variations in the cellular structures, which are simply manifestations of profound changes in the liquid portions of the blood.

In this connection we must distinguish between an increase in the proteins of the blood (hyperalbuminosis) and a decrease in their amount (hypalbuminosis). The former is observed whenever water is more rapidly withdrawn from the system, and so from the blood, than it can be supplied. Such conditions are furnished by marked diarrhea, cholera, profuse perspiration, and polyuria without extra intake of fluid. This increase in protein content is only transient and is a result of mere concentration of the blood, the proteins passing out in relatively less amount than does the water. If the above conditions are associated with true exudation, then, of course, a hypalbuminosis of transient duration will replace the hyperalbuminosis. This decrease in the amount of total proteins is observed whenever direct loss of protein from the blood occurs. Thus Becquerel and Rodier observed a diminution of the proteins in Bright's disease, cardiac edema, and puerperal fever. Hoppe-Seyler noted a loss in melanosarcoma, while Schmidt, von Jaksch, Panum and Limbeck, and Pick have reported such a condition in severe anemias and leukemias. In most severe infections, as Ewing states, the proteins are but slightly reduced. Along with hypalbuminosis we often observe a hydremia which may be referable to a direct absorption of fluid from the tissue under the influence of a hypertonic state of the blood.

In contrast to the constancy of the total protein values of the serum, we find the relationship between the albumin and globulin markedly disturbed at times. These two bodies are in reality not definite chemical compounds, but are separable, each into two distinct substances with different solubilities and precipitation constants. This fact may have great importance as further study is made, inasmuch as Pick has shown that pseudoglobulin has associated with it the antitoxins of diphtheria and of tetanus.² The normal

¹ See Schorer, *Cor.-Bl. f. schweiz. Aerzte.*, 1913, XLIII, 1523; also, Löwy, *Deutsch. Arch. f. klin. Med.*, 1914, CXV, 318; Robertson, *Jour. Biol. Chem.*, 1915, XXII, 233; Tranter and Rowe, *Jour. Am. Med. Assn.*, 1915, LXV, 1433; Reiss, *Deutsch. Arch. f. klin. Med.*, 1915, CXVII, 175; Rakuzin, *Jour. Russ. Phys.-Chem. Soc.*, 1916, XLVIII, 1251; Rowe, *Arch. Int. Med.*, 1917, XIX, 354.

² The coagulative elements of blood serum appear to be closely associated with the euglobulin fraction. See Hess, *Jour. Exper. Med.*, 1916, XXIV, 701; Berg, *Jour. Agricul. Res.*, 1917, VIII, 449.

amount of total protein being taken as 7.62 per cent., Hammarsten has shown that this percentage is made up of 3.10 per cent. of serum globulin and 4.52 per cent. of serum albumin, the relationship of globulin to albumin being as 1:1.5. This ratio is variable, running, according to Limbeck and Pick, globulin 16.9 to 38.3 per cent. of total protein, albumin 61.7 to 83.1 per cent. of the total albuminous content. Such a wide variation makes it difficult to establish any absolute relations in disease. Erben has shown that the albumin remains about normal in pernicious anemia, while the globulin is markedly reduced. The researches of Estelle, Hoffmann, Halliburton, and Mya and Viglezio show marked pathological variations not only in the total protein content, but in the so-called "protein quotient," $\frac{\text{albumin}}{\text{globulin}}$. The latter

authors conclude among other deductions that the relation of the proteins is greatly changed in disease, especially in conditions associated with transudation and exudation, in the sense that the globulins¹ are increased while the albumins are diminished. In such states globulin is seen to be relatively more resistant and less diffusible than is albumin (Gottschalk). That this view is not uniformly applicable is noted from the work of Freund who observed in the serum of nephritis a relationship of 1:11.3 between the globulin and albumin, instead of the normal 1:1.5. Ducceschi has reported an interesting observation on this point. He found that, during the period preceding the convulsions which follow thyroidectomy, a percentage increase of albumin as regards globulins obtains, while during the convulsions the reverse conditions are present.

In this discussion I have taken no account of the total protein of the whole blood. In its determination we include not only the albumin and globulin of the serum, but also the hemoglobin of the red cells, the nucleo-proteins of the white cells, and the fibrinogen of the plasma. Traces of albumose and peptone(?) have been found in pathological conditions, the former possibly in normal states, while still other nitrogenous constituents are commonly determined with the proteins.² The total protein of the blood has been given by von Jaksch as 22.62 per cent., while Limbeck states a percentage of 25. So much depends upon the method adopted for its estimation and so much on the physiological state of the blood that comparative figures are difficultly obtainable from the literature. Regarding the determination of the total protein and of the globulin and albumin, the reader is referred to the section on Urine.³

Regarding the question of the presence of peptone in the blood, as reported by von Jaksch, Freund and Obermayer, and Ludwig, much depends on the

¹ See Epstein, *Jour. Exper. Med.*, 1912, XVI, 719; Hurwitz and Meyer, *Ibid.*, 1916, XXIV, 515; Rowe, *Jour. Lab. and Clin. Med.*, 1916, I, 485; Arch. Int. Med., 1916, XVIII, 455; *Ibid.*, 1917, XIX, 499; Hurwitz and Whipple, *Jour. Exp. Med.*, 1917, XXV, 231; Hanson, *Jour. Immunol.*, 1918, III, 139. Robin, *Bull. Acad. de méd.*, 1920, LXXXIV, 51; Kahn, *Arch. Int. Med.*, 1920, XXV, 112.

² See Folin and Denis for a discussion of protein metabolism as shown by blood examinations (*Jour. Biol. Chem.*, 1912, XI, 87 and 161; *Ibid.*, 1912, XII, 141; *Ibid.*, 1913, XIV, 29).

³ For recent methods of determination of blood proteins see Albert, *Deutsch. Arch. f. klin. Med.*, 1910, CXXVIII, 280; Cullen and Van Slyke, *Jour. Biol. Chem.*, 1920, XLI, 587; Howe, *Ibid.*, 1921, XLIX, 93 and 100; Wu, *Ibid.*, 1922, LI, 33; Berg, *Jour. Lab. & Clin. Med.*, 1921, VI, 223; Henley, *Jour. Biol. Chem.*, 1922, LII, 367.

proper differentiation of the substance found. So much has been called peptone that is, in reality, albumose, that we are uncertain whether peptone was present or, if it were, whether it was not formed in the processes used or was not a postmortem product. Devoto and Wagner could not confirm the finding of true peptone when blood from the living subject was examined. It is rather strange that peptone, which reduces the coagulability of blood when added in small amounts, should not exert this power in the vessels during life were it really present. The fact, however, that peptone and albumose have both been repeatedly found in the urine in various conditions by competent observers points to the probability of the existence of these products in the blood, although they may be combined in such a way as not to be capable of easy detection. Further elaboration of our methods of detection and differentiation may clear up a much disputed field. Bywaters believes the so-called albumose of the blood to be identical with sero-mucoid.

(D) Other Nitrogenous Constituents.

Other nitrogenous bodies than those already discussed are found in the blood in normal and, in varying amounts, in abnormal conditions. Owing to the introduction of more exact methods of study, especially of the micro-chemical methods of Folin and his coworkers, extensive investigation of the non-protein nitrogenous elements of the blood has been undertaken, to the end that variations in the amount of total non-protein nitrogen as well as of the different specific nitrogenous bodies are becoming of great importance in both diagnosis and prognosis. It is unquestionably true that a study of the relationship between the urinary nitrogen factors and those of the blood gives a much more certain index of functional perversion as well as of the extent of this abnormality than does the investigation of either group of factors alone.

Total Nitrogen.

In the determination of the total nitrogen of the blood, one includes the nitrogen referable to the various protein elements as well as that derived from the different non-protein nitrogenous bodies. The method adopted in this investigation is the usual Kjeldahl method discussed on page 235, using 5 c.c. of blood to which a little potassium oxalate is added as withdrawn in order to prevent coagulation and to insure exact measurement of the volume desired.

This factor, in itself, has very little importance. Von Jaksch gives the total nitrogen of normal blood serum as 1.37 per cent., while that of the whole blood he finds is 3.62 per cent. Variations in these figures are no doubt frequent and depend upon changes in the amount of the many different elements making up the total blood nitrogen.¹

Total Non-protein Nitrogen.

While the total nitrogen referable to the protein elements of the blood shows a practically constant value, we find the non-protein elements subject to considerable variation. Although the amounts of the various non-protein nitrogen factors are relatively small as compared with those of the protein constituents, yet we find the percentage and absolute relationships between the total non-protein nitrogen, on the one hand, and the various elements of

¹ See Grafe, *Deutsch. Arch. f. klin. Med.*, 1915, CXVIII, 149; Mosenthal and Richards, *Arch. Int. Med.*, 1916, XVII, 329; Buell, *Jour. Biol. Chem.*, 1919, XL, 63.

which it is composed, on the other, showing such distinct variations from the normal in certain pathological conditions that diagnostic and prognostic deductions may be made with increased certainty. For this reason a close study of these factors is of great importance.

Method of Folin and Wu.

This method¹ is a modification of the earlier methods of Folin and Denis.² The chief reason for the introduction of this modification is the formulation of a system of blood analysis, which will permit of the determination of a maximum number of nitrogenous constituents with one precipitation of protein material. The reagents used for this purpose have been many, most of them having certain faults. Thus Folin and Denis originally used methyl alcohol followed by an alcoholic solution of zinc chlorid; later, these workers employed a solution of meta-phosphoric acid; Greenwald³ first advocated the use of 2.5 per cent. solution of trichloroacetic acid followed by kaolin and later modified his method by using a 5 per cent. solution of this acid as the precipitant; Gettler and Baker⁴ use mercuric chlorid, while Welker and Falls⁵ consider alumina cream as especially valuable for serum; recently Fischer⁶ has advised the use of uranium acetate. While the use of tungstic acid, as employed in this test, does not yield any better results, as far as the precipitation of the blood protein is concerned, than do the trichloroacetic acid of Greenwald or the meta-phosphoric acid of Folin and Denis, yet this procedure admits of the determination of practically all the non-protein nitrogenous elements of the blood and is, therefore, recommended here as the precipitant of choice.

Obtaining the Blood.

To the tip of a perfectly clean, dry serological pipet, attach the usual serological needle by means of a short piece of pure gum tubing. Drop a small pinch of powdered potassium oxalate (citrate should be avoided in this test, except in minimal amounts) into the upper end of the pipet and allow it to run down into the tip (20 mg. of the oxalate is sufficient for 10 c.c. of blood, more interferes with the later uric acid determinations). Connect the upper end of the pipet with a rubber suction tube, which is, in turn, connected with a short glass mouthpiece. Insert the needle into the median basilic vein and, by suction, fill the syringe with the blood. It is not necessary that any definite amount be drawn, as the method is adaptable to any obtainable amount of blood. It is wise, however, to draw at least 10 c.c. if possible, which will permit of duplicate determinations if desired. The blood may, then, be transferred to a clean test tube and kept in the ice box if the determination is not to be made at once.⁷

¹ Jour. Biol. Chem., 1919, XXXVIII, 81.

² Ibid., 1912, XI, 527; Ibid., 1916, XXVI, 491.

³ Ibid., 1915, XXI, 61; Ibid., 1918, XXXIV, 97.

⁴ Ibid., 1916, XXV, 210.

⁵ Ibid., 567.

⁶ Ztschr. f. physiol. Chem., 1918, CII, 266.

⁷ White and Watson (Jour. Lab. & Clin. Med., 1920, VI, 45) have shown that a sample of blood may be kept in a clean container for 24 hours without undergoing any marked decomposition.

Precipitation of Protein.

Transfer a measured amount of blood into a flask having a capacity of 15 or 20 times that of the volume taken. It is not necessary that volumetric flasks be used. For the measurement of the blood, special pipets graduated to 15 c.c. are recommended, but the usual Mohr's 10 c.c. graduated pipets are very satisfactory. Dilute the blood with 7 volumes of water and mix. With an appropriate pipet add 1 volume of 10 per cent. solution of sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$) and mix. With another suitable pipet add to the contents in the flask (with shaking) 1 volume of $\frac{2}{3}$ normal sulphuric acid. Close the mouth of the flask with a rubber stopper and give a few vigorous shakes. When the blood is properly coagulated, the color of the coagulum gradually changes from pink to dark brown. If this change does not occur, the coagulation is incomplete, due to too much oxalate or citrate. In such an emergency, add 2N. sulphuric acid, drop by drop, shaking vigorously after each addition and allowing the mixture to stand for a few minutes before adding more. Pour the mixture on a filter large enough to hold the entire contents of the flask and cover with a watch-glass. If this filtration is begun by pouring the first few c.c. of the mixture down the double portion of the filter paper and withholding the remainder till the whole filter has been wet, the filtrates are almost invariably as clear as water from the first drop. If the filtrate is not perfectly clear, the first few c.c. may have to be refiltered. As Folin and Wu state, this precipitation is so simple that no one need go astray, provided that the sodium tungstate and the $\frac{2}{3}$ normal sulphuric acid are correct. The quality of the tungstate may not be what it should be, owing to a large percentage of carbonate. This carbonate content may be determined as follows: To 10 c.c. of the 10 per cent. solution, add one drop of phenolphthalein solution and titrate with 0.1 N. hydrochloric acid. Each c.c. of the HCl corresponds to 1.06 per cent. of sodium carbonate.¹ The amount of acid required for the titration should not exceed 0.4 c.c. The amount of sulphuric acid, used in the test, is intended to set free the whole of the tungstic acid with about 10 per cent. excess (and to neutralize the carbonate usually present in commercial tungstates). A greater excess of sulphuric acid must not be used, as a large part of the uric acid will be lost by such procedure. If the filtrate, obtained as above, is to be kept for any length of time, one or two drops of toluol or xylol should be added to it.

Technic.

Introduce 5 c.c. of the protein-free blood filtrate (corresponding to 0.5 c.c. of blood) into a clean (washed with water and alcohol) and dry 75 c.c. Pyrex test-tube, which is graduated at 35 and at 50 c.c. Add 1 c.c. of the

¹ Folin (Jour. Biol. Chem., 1922, LI, 419) again calls attention to the necessity of checking up the quality of the tungstate used. See Wu (Ibid., 1920, XLIII, 189) for a study of phosphotungstic acid and allied substances.

sulphuric-phosphoric acid digestion mixture.¹ Add a dry quartz pebble or a dry glass bead and boil vigorously over a microburner until the characteristic dense acid fumes begin to fill the tube. This is usually accomplished in from 3 to 7 minutes. When the fumes are unmistakable, cut down the size of the flame so that the contents of the tube are just visibly boiling, and close the mouth of the tube with a watch-glass or a small Erlenmeyer flask. Continue the heating very gently for 2 minutes from the time the fumes begin to be unmistakable, even if the solution has become clear and colorless at the end of 20 to 30 seconds. If the oxidations are not visibly finished at the end of the 2 minutes, the heating must be continued until the solution is nearly colorless. Allow the contents to cool for 70 to 90 seconds and then add 15 to 25 c.c. of water. Cool further, approximately to room temperature, and add water to the 35 c.c. mark. Add, preferably with a pipet, 15 c.c. of Nessler's solution.² Insert a clean rubber stopper and mix. If the solution is turbid, centrifuge a portion before making the color comparison with the standard solution of ammonium sulphate (see page 239). The standard most commonly required in this test is 0.3 mg. of N, which amount should be placed in a 100 c.c. flask. Add to it 2 c.c. of the digestion mixture, about 50 c.c. of water, and 30 c.c. of Nessler solution. Fill to the mark and mix. The unknown and the standard should be Nesslerized at approximately the same time. If the standard, in the colorimeter, is set at 20 mm. for the color comparison, then 20 divided by the reading of the unknown and multiplied by 30 gives the non-protein nitrogen in mg. per 100 c.c. of blood.³

Separate Analysis of Corpuscles and Plasma.

In some cases, especially where it is desired to study the distribution of the chemical constituents between the corpuscles and plasma, it is necessary

¹ This digestion mixture is made up as follows: Mix 300 c.c. of phosphoric acid syrup (about 85% H_3PO_4) with 100 c.c. of concentrated sulphuric acid. Transfer to a tall cylinder, cover well to exclude the absorption of ammonia, and set aside for sedimentation of calcium sulphate. This is very slow, but in the course of a week or so the top part is clear and may be drawn off by means of a pipet. (It is not absolutely necessary that the calcium be removed, but it is probably safer to have it done). To 100 c.c. of the clear acid add 10 c.c. of 6 per cent. copper sulphate solution and 100 c.c. of water. Two c.c. of this solution are equivalent to 1 c.c. of the acid mixture described on page 239.

² This Nessler solution may be prepared as directed on page 239. As the mercuric iodid, now purchasable on the market, may contain insoluble impurities, which make it difficult to obtain a clear solution on the addition of potassium iodid, the following method of preparing this reagent is recommended: Transfer 150 grams of potassium iodid and 110 grams of iodine to a 500 c.c. Florence flask; add 100 c.c. of water and an excess of metallic mercury (140 to 150 grams). Shake the flask continuously and vigorously for 7 to 15 minutes or until the dissolved iodine has nearly disappeared. The solution becomes quite hot. When the red iodine solution has begun to become visibly pale, though still red, cool in running water and continue the shaking until the reddish color has been replaced by the greenish color of the double iodid of mercury and potassium. This operation requires about 15 minutes. Now separate the solution from the surplus mercury by decantation and washing with liberal quantities of distilled water. Dilute the solution and washings to a volume of 2 liters. If the cooling is begun in time, the resulting reagent is clear enough for immediate dilution with alkali. Introduce into a large bottle 3500 c.c. of 10 per cent. sodium hydrate solution, add 750 c.c. of the double iodid solution and 750 c.c. of distilled water, giving 5 liters of Nessler's solution.

³ See Bock, Jour. Biol. Chem., 1916, XXVIII, 357; Grigant and Guérin, C. R., soc. biol., Paris, 1918, LXXXI, 1139; Langstroth, Jour. Biol. Chem., 1918, XXXVI, 377; Peters, Ibid., 1919, XXXIX, 285; Bonninger, Ztschr. f. exp. Path. u. Ther., 1919, XX, 63; Feigl, Ztschr. f. exp. Med., 1919, VIII, 367; Delaby, Bull. soc. pharmacol., 1920, XXVII, 372; Stehle, Jour. Biol. Chem., 1920, XLV, 232; Feigl, Ztschr. f. exp. Med., 1921, XII, 55; Kennaway, Biochem. Jour., 1921, XV, 510; Mukai, Ibid., 516.

to make separate determinations of the composition of each. Wu¹ has recently advanced a method for such examinations.

The oxalated blood is centrifuged in graduated tubes until the volume of the corpuscles remains constant. The length of time required for this may vary. After noting the volume of the whole blood and that of the corpuscles, carefully pipet off the plasma without disturbing the corpuscles. This is best done by means of a pipet connected at the upper end with soft rubber tubing. Measure a convenient volume of the plasma, dilute with 8 volumes of water, and then add $1\frac{1}{2}$ volume each of the 10 per cent. sodium tungstate solution and $\frac{2}{3}$ N. sulphuric acid. Stopper the flask and shake. The remaining portion of the work is exactly as outlined above.

Remove the plasma that remains above the corpuscles as completely as possible. Insert a blood pipet into the corpuscular layer and take out a convenient volume. Lake it with 5 volumes of water and, after thorough rinsing of the pipet with the corpuscle solution, add 2 volumes each of the tungstate solution and the sulphuric acid. Stopper the flask and shake. The remaining steps are as given above.

Amount of Non-protein Nitrogen.

This non-protein nitrogen of the blood has been given various names in times past, among which we find "incoagulable nitrogen," "extractive nitrogen," "filtrate nitrogen," "non-dialyzable nitrogen," "rest," "waste," or "retention nitrogen." Many figures have been given by various workers for this factor, but these depend, to a large extent, on the method employed for its determination as well as upon the state of digestion at the time the blood is drawn for examination. It has been clearly demonstrated that the "non-protein nitrogen of the blood does rise and sink like a tide with reference to absorption from the digestive tract." This "digestive rise" is, of course, not a very great one, but it is sufficient to necessitate a slightly variable figure for the normal value of non-protein nitrogen of the blood. Folin and Denis consider the normal value to be between 22 and 26 mg. per 100 c.c. of blood. During digestion the rise is about 4 mg., so that we may consider a normal non-protein figure to be below 30 mg. per 100 c.c. of blood.

As the kidney is the great regulator of the composition of the blood, maintaining a practically constant level of non-protein nitrogen, it is in disorders of this organ, especially, that most is to be expected from a study of the variations in the non-protein nitrogen of the blood. In the interpretation of an increase in the non-protein nitrogen in nephritis Mosenthal considers that four factors must be regarded: (1) retention of nitrogen by an insufficient kidney; (2) inspissation of blood due to loss of water; (3) increase of protein catabolism; and (4) the chemical combination in which the non-protein nitrogen exists in the blood. In cases of high fever, associated with marked toxogenic decomposition of protein, it might be rational to suppose that these decomposition products would find their way into the blood current and increase the non-protein nitrogen of the blood. As a matter of fact we do find in pneumonia a considerable increase, as a rule, reaching its maximum toward

¹ Jour. Biol. Chem., 1922, LI, 21.

the crisis but bearing no relation to the time at which resolution takes place. In very few of the other fevers, however, has a rise of any importance been noted.

Numerous workers have shown that, in the majority of cases, the non-protein nitrogen increases with an increasingly severe nephritis. In many cases of nephritis, however, the non-protein nitrogen as well as the urea fall within normal limits. It is probably true that, in both chronic interstitial and chronic diffuse nephritis, cases without symptoms of uremia show normal or only moderately elevated values. In cases showing considerable hypertension, the non-protein nitrogen may be much increased. In cases tending toward uremia or showing actual uremia, the values are markedly increased, reaching in some cases as high as 350 mg. per 100 c.c. Amounts of 100 mg. or over per 100 c.c. are rarely seen in conditions other than uremia, so that this factor assumes great importance in diagnosis. Further, the prognostic value of this examination is shown in the fact that patients with high non-protein nitrogen do not, as a rule, survive for a very long period. Another valuable point in the study of this factor is that it furnishes a guide to the proper diet to be allowed nephritics, as cases with high retention require restriction of protein. In chronic passive congestion of the kidneys there is little or no retention, while in chronic lead poisoning, which is so often associated with granular kidneys, considerable retention may be observed, but the figure never reaches that shown in uremic conditions. In the eclampsias of pregnancy we seldom find a marked increase in the non-protein nitrogen, at least so far as reported cases are to be considered. This slight retention, in obscure pregnancy, may serve as a point in differential diagnosis from uremia.¹

¹ See Folin and Farmer, *Jour. Biol. Chem.*, 1912, XI, 493; Folin and Denis, *Ibid.*, 87, 161, 503 and 527; *Ibid.*, 1912, XII, 141 and 253; Folin and Lyman, *Ibid.*, 259; Löwy, *Ztschr. f. physiol. Chem.*, 1912, LXXIX, 349; Folin and Denis, *Jour. Biol. Chem.*, 1913, XIV, 29; *Ibid.*, 1913, XVII, 487 and 493; Michaud, *Cor.-Bl. f. Schweiz. Aerzte*, 1913, XLIII, 1474; Farr and Austin, *Jour. Exper. Med.*, 1913, XVIII, 228; Karsner and Denis, *Ibid.*, 1914, XIX, 259; Folin, Denis and Seymour, *Arch. Int. Med.*, 1914, XIII, 224; Agnew, *Ibid.*, 485; Frothingham and Smillie, *Ibid.*, 1914, XIV, 541; Tileston and Comfort, *Ibid.*, 620; Mosenthal, *Ibid.*, 844; Gulick, *Jour. Biol. Chem.*, 1914, XVIII, 541; McLean and Selling, *Ibid.*, 1914, XIX, 31; Pribram, *Zentralbl. f. inn. Med.*, 1914, XXXV, 153; Hertz, *Wien. klin. Wchnschr.*, 1914, XXVII, 323; Austin and Miller, *Jour. Am. Med. Assn.*, 1914, LXIII, 944; Farr and Krumbhaar, *Ibid.*, 2214; Farr and Williams, *Am. Jour. Obs.*, 1914, LXX, 614; *Am. Jour. Med. Sc.*, 1914, CXLVII, 556; Frothingham, *Ibid.*, 1915, CXLIX, 808; Plass, *Am. Jour. Obs.*, 1915, LXXI, 608; Foster, *Arch. Int. Med.*, 1915, XV, 356; Fitz, *Ibid.*, 524; Hopkins and Jonas, *Ibid.*, 964; Schultz and Pettibone, *Am. Jour. Dis. Child.*, 1915, X, 206; Tileston and Comfort, *Ibid.*, 278; Hohweg, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1915, XXVIII, 459; *Med. Klin.*, 1915, XI, 331; Bock and Benedict, *Jour. Biol. Chem.*, 1915, XX, 47; Myers and Fine, *Ibid.*, 391; Greenwald, *Ibid.*, 1915, XXI, 61; Harding and Warneford, *Ibid.*, 69; Folin, *Ibid.*, 195; Taylor and Hulton, *Ibid.*, 1915, XXII, 63; Taylor and Lewis, *Ibid.*, 71; Pepper and Austin, *Ibid.*, 81; Folin and Denis, *Ibid.*, 1915, XXII, 321; Woods, *Arch. Int. Méd.*, 1915, XVI, 577; Christian, Frothingham, O'Hare and Woods, *Am. Jour. Med. Sc.*, 1915, CL, 655; Wolf and Gutmann, *Deutsch. Arch. f. klin. Med.*, 1915, CXVIII, 174; Bang, *Biochem. Ztschr.*, 1916, LXXII, 104, 119, 129, 139, and 146; Pozzilli, *Policlinico*, 1916, XXIII, 33; Scheel, *Ugesk. f. Laeger*, 1916, LXXVIII, 493 and 541; Slemmons and Morris, *Bull. Johns Hopk. Hosp.*, 1916, XXVII, 343; Cooke, Rodenbaugh, and Whipple, *Jour. Exper. Med.*, 1916, XXIII, 717; Karsner, *Jour. Lab. and Clin. Med.*, 1916, I, 910; Leopold and Bernhard, *Am. Jour. Dis. Child.*, 1916, XI, 432; Rosenberg, *Berl. klin. Wchnschr.*, 1916, LIII, 1314; Minsk and Sauer, *Am. Jour. Dis. Child.*, 1917, XIII, 397; Mosenthal and Hiller, *Jour. Urol.*, 1917, I, 75; Whipple and Van Slyke, *Jour. Exper. Med.*, 1918, XXVIII, 213; Feigl, *Arch. f. exp. Path. und Pharm.*, 1919, LXXXIII, 299, 317, and 335; Brun, *Acta Med. Scand.*, 1919, LII, 367; Slemmons, *Canad. Med. Quart.*, 1919, II, 447; Hisanobu, *Am. Jour. Physiol.*, 1919, L, 357; Chapin and Myers, *Am. Jour. Dis. Child.*, 1919, XVIII, 555; Myers and Killian, *Am. Jour. Med. Sc.*, 1919, CLVII, 674; Weston, *Arch. Neurol. & Psychiat.*, 1920, III, 147; Wells,

Urea.

This is, of course, the most important of the single elements which make up the total non-protein nitrogen of the blood. Its importance lies in the fact that its accumulation in the blood runs parallel to that of the total non-protein nitrogen and, hence, is of much diagnostic and prognostic value.

Method of Van Slyke and Cullen.

This method is an adaptation of the same method discussed in the section on urine. Three c.c. of fresh blood or other body fluid are measured with an accurate pipet and run into a 100 c.c. heavy Jena test-tube containing 1 c.c. of 3 per cent. potassium citrate solution to prevent clotting. Five-tenth of a c.c. of urease solution (prepared by the method of Van Slyke and Cullen, see *Urine*, page 247) and two or three drops of caprylic alcohol are added. Allow this mixture to stand 10 minutes and then add 5 c.c. of saturated potassium carbonate solution. Drive off the generated ammonia by means of an air current into 25 c.c. of N/100 hydrochloric acid. Titrate back the excess of acid with N/100 sodium hydrate solution, using methyl red as an indicator. Each c.c. of N/100 acid neutralized indicates 0.00014 gram nitrogen. As 3 c.c. of blood were used in this process, we find that each c.c. of N/100 acid neutralized is equivalent to 0.00467 gram of nitrogen referable to urea or to 0.01 gram of urea per 100 c.c. of blood. As fresh blood contains so little ammonia, as such, it may be disregarded in this process.

Method of Folin and Wu.

This method¹ is a part of the system of blood analysis arranged by these workers and is, in reality, a modification of the method of Van Slyke and Cullen just discussed. It consists of the decomposition of the urea in the blood filtrate to ammonia by the use of jack bean urease in the presence of a new phosphate solution and the distillation or aeration of the ammonia into a known solution of dilute acid.

Technic.

Transfer 5 c.c. of the tungstic acid blood filtrate (previously discussed under Non-protein Nitrogen on page 497) to a clean and dry Pyrex ignition tube (capacity about 75 c.c.).² Add to the blood filtrate two drops of the

Arch. Int. Med., 1920, XXVI, 443; Gettler and Lindeman, *Ibid.*, 453; Williams, *Ibid.*, 748; Cummer, *Jour. Lab. & Clin. Med.*, 1920, V, 257; Myers, *Ibid.*, 343 and 418; Chace and Myers, *Jour. Am. Med. Assoc.*, 1920, LXXIV, 641; Foster, *Ibid.*, 1921, LXXXVI, 281; Williams, *Arch. Int. Med.*, 1921, XXVIII, 426; Rakestraw, *Jour. Biol. Chem.*, 1921, XLVII, 565; Killian and Sherwin, *Am. Jour. Obs. & Gyn.*, 1921, II, 6; Caldwell and Lyle, *Ibid.*, 17; Vaughan and Morse, *Arch. Surg.*, 1921, III, 405; MacLean and Boyd, *Brit. Med. Jour.*, 1921, II, 425; Host and Hatlehol, *Quart. Jour. Med.*, 1921, XV, 43; Jamieson, *Arch. Dermatol. & Syphilol.*, 1921, IV, 622; Barat and Hetenyi, *Deutsch. Arch. f. klin. Med.*, 1922, CXXXVIII, 154; Weiss and Garner, *Jour. Lab. & Clin. Med.*, 1922, VII, 229; Wilhelms, *Ibid.*, 622; Ponder, *Biochem. Jour.*, 1922, XVI, 368.

¹ *Jour. Biol. Chem.*, 1919, XXXVIII, 81; see, also, Folin and Denis, *Ibid.*, 1912, XI, 527; *Ibid.*, 1916, XXVI, 505; Landolph, *Sem. Med.*, 1916, XXIII, 337; Hulton-Frankel, *Jour. Lab. and Clin. Med.*, 1918, III, 548; Bahlmann, *Nederl. Tijdschr. v. Geneesk.*, 1920, I, 473; Steinfield, *Jour. Am. Med. Assoc.*, 1920, LXXV, 473; Gad-Andresen, *Jour. Biol. Chem.*, 1922, LI, 373.

² One should never use for this decomposition the Pyrex tubes employed in the non-protein determinations, unless they are first washed with nitric acid to remove the mercury films, which are prone to collect on the walls of the tubes. After treatment with the nitric acid, wash thoroughly with water and follow by alcohol and thorough drying. Mercury prevents the action of urease and must be removed.

sodium pyrophosphate solution, whose composition is given below.¹ Then add 0.5 to 1 c.c. of the jack bean urease solution,² and immerse the test tube in a beaker of warm water and leave it there for 5 minutes. The temperature of the water is not especially important, but should not exceed 55° C. If one prefers not to use the hot water bath, room temperature may be employed allowing the digestion to continue for 10 to 15 minutes or longer. The ammonia formed in this process may be conveniently and quickly distilled into 2 c.c. of 0.05 N hydrochloric acid contained in a second test-tube, which need not be as heavy as the Pyrex tube and should be graduated at 25 c.c.³ Add to the hydrolyzed blood filtrate a dry pebble, 2 c.c. of saturated borax solution and a drop or two of paraffin oil; firmly insert the rubber stopper carrying both delivery tube and receiver (care being of course taken that the delivery tube dips below the acid solution in the receiver), and boil moderately fast over a microburner for 4 minutes. The size of the flame should never be cut down during the distillation, nor should the boiling be so brisk that the emission of steam from the receiving tube begins before the end of 3 minutes. At the end of 4 minutes slip off the receiver from the rubber stopper and lower the receiver in such a way that the delivery tube rests along the side of the receiver about midway between the 25 c.c. graduation mark and the top of the receiving tube. Continue the distillation for 1 minute more and rinse off the lower outside part of the delivery tube with a little water. Cool the distillate with running water, dilute to about 20 c.c. and add 2.5 c.c. of the Nessler solution discussed on page 498. Fill to the

¹ The composition of the phosphate solution used is as follows. To 140 grams of sodium pyrophosphate (U.S.P.) add 20 grams of glacial phosphoric acid and make up to 1 liter. Instead of this solution, one may use a molecular ortho-phosphate solution, consisting of a mixture of molecular monosodium phosphate and molecular disodium phosphate solutions in the proportion of $\frac{1}{3}$ of the former to $\frac{2}{3}$ of the latter.

² The urease solution is prepared as follows: Transfer to a 200 c.c. flask or bottle about 3 grams of permutit powder. Wash this by decantation, once with 2 per cent. acetic acid, then twice with water. Add to the moist permutit in the flask 100 c.c. of 30 per cent. alcohol. Then introduce 5 grams of jack bean meal (which may be obtained from the Arlington Chemical Co., Yonkers, N. Y.) and shake for 10 minutes. Filter and collect the filtrate in three or four different small clean bottles. Set one aside for immediate use, as it will remain serviceable for at least 1 week at ordinary room temperature if not exposed to direct sunlight. Place the other bottles in the ice box, where they will remain active for 3 to 5 weeks. The addition of the buffer solutions (phosphate mixtures) is essential when this solution is used, as the decomposition of urea is never dependable otherwise. This action is, according to Folin, twofold: they not only accelerate the decomposition of the urea, but they prolong the acting period of the urease to a great extent.

³ The distilling apparatus recommended by Folin and Wu consists of the original Pyrex test-tube, to which is attached a second test-tube, which serves as a receiver. This latter is held in place by means of a rubber stopper in the side of which has been cut a fairly deep notch to permit the escape of air (and some steam). The rubber stopper, serving as a holder for the receiver, fits quite loosely to the delivery tube by means of which the test-tubes are connected. In the beginning of the distillation, the delivery tube must dip below the acid in the receiver; near the end this delivery tube is raised about midway between the graduation mark on the test-tube and the top of the tube. Watson and White (Jour. Biol. Chem., 1921, XLV, 405) have modified this distilling apparatus slightly, in order to overcome the frothing during distillation. An ordinary 25 c.c. pipet, with a bulb in the center, is bent downward at an angle of about 45° and a second bend is made in the tube so that its two arms are parallel. A number of small holes are blown in the side and a constriction is made at the end of the tube from which the distillation proceeds. The large bubbles of foam, in passing through the numerous small holes, are broken up and the bulb in the upper bent portion of the tube further safeguards any possibility of liquid being carried over into the receiver. Youngburg (Jour. Lab. and Clin. Med., 1922, VII, 552) uses open delivery tubes in this distillation.

25 c.c. mark with water and compare in the colorimeter with a standard containing 0.3 mg. of N (in the form of ammonium sulphate) in a 100 c.c. flask and Nesslerized with 10 c.c. of the Nessler solution. Both the standard and unknown should always be Nesslerized as nearly simultaneously as practicable. Multiply 20 (the height of the standard in mm.) by 15 and divide by the colorimetric reading to get the urea nitrogen per 100 c.c. of blood. To convert the nitrogen values into terms of urea, multiply the N by 2.143.

Should one prefer to use the process of aeration proceed as follows: Decompose the urea of the blood filtrate as described above. Add 1 or 2 c.c. of 10 per cent. sodium hydroxid solution and aspirate the ammonia (as described on page 253) into a test-tube graduated at 25 c.c. and containing 2 c.c. of 0.05 N hydrochloric acid. Precaution must be taken that the rubber tubing connections are well washed with water before being used, as new tubing is coated with talcum powder which may contain ammonia. The method, after aeration, is as above described.

Further, if one prefers, the autoclave method may be adopted. To 5 c.c. of blood filtrate in a 75 c.c. Pyrex test-tube is added 1 c.c. of normal acid; the mouth of the tube is covered with tin-foil, and the test-tube with contents is placed in an autoclave and heated at 150°C. for 10 minutes. Allow the autoclave to cool below 100°C. before opening. Now distill off the ammonia as given above, using 2 c.c. of 10 per cent. sodium carbonate solution instead of the borax employed in the first process discussed. If desired, the ammonia may be driven off by aeration as usual. The procedure from this point is the same as given above.

Amount of Urea.

There is much confusion in the literature regarding the amount of urea in the blood, as the methods used have been numerous and the urea has been reported both in terms of urea and of urea nitrogen. The older methods must be regarded as inaccurate, so that figures obtained from them cannot be accepted at the present time. To obtain comparative figures for these different factors, multiply the urea by 0.467 to get the urea nitrogen, or multiply the urea nitrogen by 2.143 to derive the urea.

Folin and Denis, in their analysis of the blood of sixteen normal individuals, report that the urea nitrogen varies only between 11 and 13 mg. (approximately 24 to 28 mg. of urea) per 100 grams of blood. As these specimens of blood were collected in the morning 3 to 6 hours after breakfast, the results are to be considered as essentially those from a fasting person. During the digestion of a full meal containing meat, these values are probably increased by 2 to 3 mg. or more of urea nitrogen. Other workers, using the same method, report higher figures for the normal urea nitrogen, the values running in some cases as high as 20 to 25 mg. in 100 c.c. of blood. These higher figures are perhaps above the true normal values, but they represent the usual findings in the everyday run of clinical material. The urea nitrogen normally constitutes about 50 per cent. of the total non-protein nitrogen. In some cases it may be a trifle higher, but almost never is it found to be lower. During digestion the percentage relationship is, of course, increased.

In a general way it is to be expected that variations in the amount of urea nitrogen will parallel those of the total non-protein nitrogen. As urea is excreted largely through the kidney, serious disturbance of the renal parenchyma should be associated with retention of urea. As a matter of fact, it has been found that in severe cases of nephritis, accompanied with symptoms of uremia, retention of urea is marked, reaching in some cases as high as 250 mg. urea nitrogen per 100 c.c. of blood and constituting 80 per cent. or more of total non-protein nitrogen. It is evident, therefore, that marked retention of urea is a positive indication of almost complete renal insufficiency. A large part of the kidney parenchyma may be pathological and yet no retention of urea occur, providing the remaining renal tissue is sufficient to take care of the ordinary systemic requirements. In other words, cases either of interstitial or diffuse nephritis may show little or no retention of urea. Should urea retention become marked in such cases, uremia is impending owing to the excessive work put upon the remaining functioning renal cells. Chronic passive congestion of the kidney almost never shows urea retention. Further, one kidney may be so extensively diseased as to be incapable of functioning and yet no retention of urea occur, as the vicarious activity of the remaining kidney prevents any accumulation. For this reason, this determination of blood urea can have no importance in the study of unilateral renal diseases.

A number of French investigators have advocated a comparison of the concentration of urea as excreted in the urine with its concentration in the blood, believing that a very close relationship exists. Ambard, as a result of his studies, has formulated two general laws with reference to this point as follows: (1) When the concentration of urea in the urine is constant, the quantity of urea excreted in the urine varies proportionately to the square of the concentration of urea in the blood. (2) When the concentration of urea in the blood remains constant, the quantity excreted in the urine varies inversely as the square root of the concentration in the urine. From these laws, corrected for the weight of the individual (70 kilos being the standard) and the average concentration of urea in the urine (25 grams per liter), Ambard formulated his "*uremic constant*" or "*coefficient*," which is as follows:

$$K \text{ (constant)} = \frac{Ur}{\sqrt{D \times \frac{70}{P} \sqrt{\frac{C}{25}}}}$$

in which Ur = grams of urea per liter of blood; D = grams of urea in 24-hour specimen of urine; P = weight of patient in kilos; C = concentration or grams of urea per liter of urine.

Normally this coefficient of Ambard is 0.06 to 0.09, being practically unaffected by variations in diet as is the amount of urea in the blood. Where the amount of urine excreted is very low, this constant tends toward higher values. In cases of severe nephritis a large increase in this coefficient is observed associated with a relative increase in the concentration of urea in the blood and a decrease in the elimination. It would seem, therefore, that this

coefficient of Ambard might be of considerable value in the study of cases showing little actual retention of urea, as far as the absolute amount of the urea is concerned, but in which actual evidence of nephritis is present. In other words, may not a renal insufficiency be indicated by this coefficient, when the amount of urea nitrogen is practically normal? Although we have been somewhat loath to accept the conclusions of Ambard and his coworkers, recent studies, especially by McLean and Selling, would indicate that an increased coefficient may be found in nephritic cases, which do not show any appreciable retention of urea. In the severe types of nephritis, which are tending toward uremia, this coefficient will be markedly increased, just as are the total non-protein nitrogen and urea.

As McLean points out, the determination of the amount of urea nitrogen, or of the total nonprotein nitrogen in the blood, is not entirely dependable as a measure of excretory activity. The normal concentration of urea in the blood is not fixed, but varies within wide limits in response to variations in nitrogen and fluid intake. As a matter of fact, the larger proportion of individuals with disturbed urea function have concentrations of urea in their blood which are below the upper limits in normal individuals, so that the line of demarcation between normal and abnormal subjects is not sharp. Blood urea figures alone, therefore, may call attention only to the more serious instances of disturbed function. In his work McLean has extended the laws of Ambard and introduced a relatively simple method for making clinical estimations of the state of the function which has to do with excretion of urea. "It is important to state that investigations concerning urea excretion are especially valuable in the study of renal disease. For although the various functions of excretion by the kidney appear to be quite specific, so that no one test can be regarded as a measure of total renal function, nevertheless, the information obtained from a study of the urea function appears to be more general than that of any other."

The "*Index of Urea Excretion*," as originated by McLean¹ is based on an ideal normal Ambard's coefficient of 0.08. Deficiency in urea function results in higher values. These higher values are due to a change in the ratio which exists between the concentration of urea in the blood and the rate of its excretion. Such findings indicate deviation from the normal, but do not measure the extent of the functional change which is responsible for such deviation. In observations in human subjects it is not possible to keep conditions constant, but it is possible, by an application of Ambard's laws, to measure the influence of their variations on the rate of excretion of urea. The ideal normal rate of excretion, under the conditions found in McLean's studies, at any time, is given a value of 100. The index then expresses, in direct percentage, the rate of excretion found, in terms of the rate of excretion that a normal individual would develop under the same conditions as to concentration in the blood, concentration in the urine, and body weight. Ambard's laws depend on the constant relationship of four variables: (1) the concentration of urea in the blood; (2) the concentration of urea in the

¹ Jour. Exper. Med., 1915, XXII, 212 and 366; Jour. A. M. A., 1916, LXVI, 415.

urine; (3) the rate of urinary excretion, which together with (2) gives the rate of urea excretion; and (4) the weight of the individual. When variables which give an Ambard's coefficient of 0.08 (the figure chosen as the ideal normal) are substituted in the Ambard formula, the constant must have a value of 8.96 to give an index of 100. The new formula of McLean would then be

$$\text{Index of Urea Excretion} = \frac{D\sqrt{C \times 8.96}}{\text{Wt} \times \text{Ur}^2} = 100 \text{ (normal value).}$$

In this formula D = Grams urea excreted per 24 hours; C = Grams urea per liter urine; Ur = Grams urea per liter blood; Wt. = Body weight of individual in kilograms.

The rate of excretion is not actually determined for 24 hours, but for a shorter period, usually 72 minutes ($\frac{1}{20}$ of 24 hours), the calculation being made on a basis of grams per 24 hours. It is important to remember that it is not the actual twenty four hour excretion of urea that is essential, but the rate of excretion at the time of observation. McLean's procedure in the determination of this index is as follows: The patient is given from 150 to 200 c.c. of water in order to insure a free flow of urine. One-half hour later he voids urine, in order to start with the bladder empty. The patient may be catheterized if necessary. The time of voiding is recorded to within 1 minute. About 36 minutes later, from 7 to 10 c.c. of blood are withdrawn in the usual way and a few mg. of potassium oxalate added to prevent clotting. At the end of 72 minutes from the first voiding, the bladder is again emptied and the urine accurately measured. The patient takes no food or water during this period. If it is desired to carry out a simultaneous phenolsulphonephthalein test, one may adopt the 1 or 2 hour period, instead of the 72 minute one, half of the measured urine being used for the phenolsulphonephthalein reading, and the other half for the necessary analyses in connection with this method, which are determination of blood urea, urine urea, and urine ammonia by methods previously outlined. Knowing these factors and the weight of the naked individual within 1 kilogram, the variables are inserted in the formula and the index calculated. This has been simplified by McLean, by the introduction of a special modified 10 inch slide-rule (obtainable from Keuffel and Esser Co., 127 Fulton St., New York), which permits a direct reading of the index.

An index below 80 is to be considered as abnormal, though not necessarily seriously so. In renal disease, an index below 50 is indicative of a considerable degree of impairment of functional ability. The lower the index, the greater the impairment to the kidney. However, a low index may be only temporary, as in passive congestion of heart failure or in acute nephritis. When observations are made under the proper conditions, values for the index of less than 80 should never be found in normal individuals. Values above 100 are the rule and values up to 180 and 200 may occur. Much higher values may be obtained under certain pathologic conditions, associated with an increase in the rate of urea excretion. The figure obtained for the blood urea will give some indication as to the probable index, as low blood urea is

associated with a high index and vice versa. As McLean points out, the application of the index is seriously interfered with when water excretion is greatly diminished, so that he make it a general rule not to attempt to apply this index when a rate of urinary outflow equal to at least 500 c.c. in 24 hours cannot be attained.

Addis and Watanabe conclude, from their work, that factors other than the concentration of the blood urea must commonly intervene in the process of urea excretion. They call attention to our lack of definite knowledge of the factors governing the excretion of urea by the kidneys. Lewis, also, finds that the normal variations are rather wide and are subject to many disturbing influences. McLean, in more recent work, concludes that urea retention, in the sense of a relatively increased concentration in the blood, is the result of increased resistance to the excretion of urea through the kidneys. The numerical value of Ambard's coefficient changes in urea retention, but the relation of the variable factors to one another remains otherwise unchanged. In certain individuals, with otherwise normal findings in regard to urea excretion, an unusual degree of constancy, to which McLean applies the term "fixation," has been found in the numerical results obtained by the application of Ambard's laws. These individuals are regarded as probably abnormal, but the pathological significance of the fixation has not been determined.

As the liver is concerned to a considerable extent with the formation of urea, we might expect to find in cases of marked hepatic insufficiency a reduction in the amount of urea in the blood, just as we do in the urine. However, it is to be remembered that the liver is not wholly responsible for the conversion of ammonium salts and amino acids into urea, as Folin, Van Slyke and others have shown, so that it must be regarded as possible that a practically normal urea finding may obtain in the case of the blood and the urinary output be relatively much diminished. Further, even in extensive liver disease, such as cirrhosis and carcinoma, sufficient hepatic parenchyma may remain to continue the normal function of the liver. Recent work by Farr and Krumbhaar would seem to indicate that the urea nitrogen of the blood is actually not diminished in hepatic disease in contradistinction to the findings reported by various French workers.¹

¹ With reference to various points raised in this section on urea see Ambard, *Compt. rend. Soc. de Biol.*, 1910, LXIX, 411 and 506; *Assoc. franc. d'urolog.* Proc.-Verb., 1912, XV, 518; *Bull. et mém. Soc. méd. des hôp. de Paris*, 1913, XXXVI, 765; Widall and Javal, *Ibid.*, 1911, XXXII, 627; Achard, *Le rôle de l'urée en pathologie*, Paris, 1912; Ambard and Weill, *Jour. de Physiol. et de Path. gén.*, 1912, XIV, 753; Folin and Denis, *Jour. Biol. Chem.*, 1912, XI, 87; *Ibid.*, 1913, XIV, 29; *Ibid.*, 1913, XV, 493; Rowntree and Fitz, *Arch. Int. Med.*, 1913, XL, 258; Farr and Austin, *Jour. Exper. Med.*, 1913, XVIII, 228; Courmont, Boudol, Savy and Blanc-Perduet, *Bull. et mém. Soc. méd. d. hôp. de Paris*, 1913, XXXV, 259; Morel and Mouriquand, *Ibid.*, 266; Chauffard, *Ibid.*, 273; Brodin, *Compt. rend. Soc. de Biol.*, 1913, LXXIV, 26; Legueu, Ambard and Chabanier, *Arch. urol. Clin. de Necker*, 1913, I, 275; Legueu, *Jour. d'Urol.*, 1914, V, I; Kholzoff, Russky Vrach, 1914, XIII, 465; Studzinski, *Ibid.*, 678; Widall, Weill and Radot, *Presse méd.*, 1914, XXII, 409 and 565; *Jour. d'Urol.*, 1914, V, 681; Tschertkoff, *Dutsch. med. Wchnschr.*, 1914, XL, 1713; Marshall and Davis, *Jour. Biol. Chem.*, 1914, XVIII, 53; McLean and Selling, *Ibid.*, 1914, XIX, 31; Folin, Denis and Seymour, *Arch. Int. Med.*, 1914, XIII, 224; Agnew, *Ibid.*, 485; Tileston and Comfort, *Ibid.*, 1914, XIV, 620; Farr and Krumbhaar, *Jour. Am. Med. Assn.*, 1914, LXIII, 2214; Underhill, *New York Med. Jour.*, 1915, CII, 662; Klinkert, *Nederl. Tijdschr.*

Uric Acid.

Like many substances formed outside the kidneys but excreted by them in fairly large quantities, uric acid occurs in the blood in traces too small to yield distinct reactions and quantitative results with the usual tests as applied to urine. The introduction of newer methods enables us to study much more closely the question of the normal uric acid values as well as the pathological variations.

Method of Benedict.

This method¹ is a modification of that of Folin and Macallum and, also, of the later one of Folin and Denis. This consists in substituting a rapid and convenient procedure in place of the rather troublesome decomposition of the uric acid compound, with hydrogen sulphid by dissolving the precipitated uric acid with potassium cyanide solution.

Technic.

Ten to 20 c.c. of blood are withdrawn from the median basilic vein by means of an accurately graduated syringe, the barrel of which contains a little powdered potassium oxalate to prevent clotting. This blood is then added to 5 volumes (50 to 100 c.c.) of boiling 0.01 N acetic acid in a casserole and the mixture heated to boiling for a moment. The casserole is then removed from the flame and 100 or 200 c.c. of boiling water (depending on the original volume of blood taken) are added. The mixture is then poured upon a folded filter, and the residue washed with 50 c.c. of boiling water (heated in the same casserole in which the original coagulation took place).

v. Geneesk., 1915, II, 1567 and 1658; Foster, Tr. Assoc. Am. Phys., 1915, XXX, 305; Grossi, Policlinico, 1916, XXIII, (sez. chir.), 41; Kinney, N. Y. Med. Jour., 1916, CIV, 208; Payan and Mattei, C. R. soc. biol., Paris, 1916, LXIX, 910; Schwartz and McGill, Arch. Int. Med., 1916, XVII, 42; Hewlett, Gilbert, and Wickett, *Ibid.*, XVIII, 637; Jones and Austin, Am. Jour. Med. Sc., 1916, CLII, 560; Addis and Watanabe, Jour. Biol. Chem., 1916, XXIV, 203; *Ibid.*, XXVII, 249; *Ibid.*, XXVIII, 251; *Ibid.*, 1917, XXIX, 391 and 399; Arch. Int. Med., 1917, XIX, 507; Lewis, *Ibid.*, 1; Watanabe, Am. Jour. Med. Sc., 1917, CLIV, 76; Nakagawa, Brit. Jour. Surg., 1917, IV, 386; Smith, Jour. A. M. A., 1917, LXVIII, 278; Pearce, Jour. Lab. and Clin. Med., 1917, II, 590; Goto, Jour. Exper. Med., 1917, XXV, 603; McLean, *Ibid.*, XXVI, 181; Stengel, Austin, and Jonas, Arch. Int. Med., 1918, XXI, 313; Frissell and Vogel, *Ibid.*, XXII, 56; Frothingham, *Ibid.*, 74; Kast and Wardell, *Ibid.*, 581; Kast and Killian, Proc. Soc. Exp. Biol. and Med., 1919, XVI, 141; Myers and Fine, Jour. Biol. Chem., 1919, XXXVII, 239; Reimann and Hartman, Am. Jour. Physiol., 1919, L, 82; McLean and De Wesselow, Quart. Jour. Med., 1919, XII, 347; Albert, Biochem. Ztschr., 1919, XCIII, 89; Merklen and Kudelski, Bull. et. mem. soc. hôp. de Paris, 1919, XXI, 604; Ameuille, Presse Méd., 1919, XXVII, 189; Rouzaud, C. R. soc. biol. Paris, 1919, LXXXII, 727; Bevier and Shevsky, Am. Jour. Physiol., 1919, L, 191; Rappelye Jour. Nerv. & Ment. Dis., 1919, XLIX, 130; Cordonnier, Bull. sci. pharmacol., 1919, XXVI, 462; Peters, Deutsch. Arch. f. klin. Med., 1919, CXXXIX, 253; Dufour and Semelaigne, Bull. et. mem. Soc. Méd. des Hôp. de Paris, 1920, XLIV, 58; Apert and Vallery-Radot, *Ibid.*, 937; Wordley, Quart. Jour. Med., 1920, XIV, 88; Louria, Arch. Int. Med., 1921, XXVII, 620; Killian and Kast, *Ibid.*, 1921, XXVIII, 813; Jacobson and Edwards, Am. Jour. Med. Sc., 1920, CLIX, 833; Manchege, Cronica Med., 1920, XXXVII, 304; Velasquez, *Ibid.*, 1921, XXXVIII, 11; Guggenheimer, Deutsch. Arch. f. klin. Med., 1921, CXXXVII, 159; Rosenberg, Mitteil. a. d. Grenzgeb. d. Med. u. Chir., 1921, XXXIV, 162.

With reference to methods for determination of urea in blood see Folin and Pettibone, Jour. Biol. Chem., 1912, XI, 507; Folin and Denis, *Ibid.*, 527; Marshall, *Ibid.*, 1913, XIV, 283; *Ibid.*, 1913, XV, 287 and 495; Van Slyke and Cullen, *Ibid.*, 1914, XIX, 211; Jour. Am. Med. Assn., 1914, LXII, 1558; Olivieri, Riv. osped., 1914, IV, 221; Kristeller, Ztschr. f. exper. Path. u. Therap., 1914, XVI, 496; Siebeck, Deutsch. Arch. f. klin. Med., 1914, CXVI, 58; Neumann, Biochem. Ztschr., 1915, LXIX, 134; Combe and Levi, Rev. med. de la Suisse Rom., 1915, XXXV, 413.

¹ Jour. Biol. Chem., 1915, XX, 629.

The total filtrate is now transferred to a casserole and boiled rapidly down to a volume of about 25 c.c. This solution is poured into a small flask roughly marked to indicate a volume of 50 c.c. The contents of the casserole are washed quantitatively into the flask with the help of two or three portions of water, heating the water to vigorous boiling, and rubbing the sides of the casserole with a rubber-tipped stirring rod each time. The total volume in the flask should not exceed 50 c.c. after addition of the washings. The turbid solution in the flask is now cooled thoroughly under running water and 2 c.c. of colloidal iron solution (Merck's "Dialyzed Iron," 5 per cent. solution) are added while the flask is gently rotated. The mixture is then filtered through a small folded filter into a 100 c.c. Jena Florence flask, the residue on the filter being twice washed with distilled water. The filtrate obtained here should be clear and colorless. The solution is now boiled down to a volume of 1 to 2 c.c., then carefully poured into a small centrifuge tube, and the flask washed out with three portions of water (1 to 2 c.c. each), heating each to boiling in the flask and shaking thoroughly prior to transferring it to the centrifuge tube. The contents of the tube, which should have a volume of 5 to 10 c.c., are now cooled and treated with 20 drops of ammoniacal silver magnesium solution.¹ The contents of the tube are mixed thoroughly with the help of a narrow stirring rod, and the tube is then centrifuged for 1 or 2 minutes. The supernatant fluid is then poured off as completely as possible, and to the residue in the tube are added one or two drops of 5 per cent. potassium or sodium cyanid solution and the mixture is thoroughly stirred for a moment. A few drops of water are then added and the mixture is again stirred. One or two c.c. of the uric acid reagent of Folin and Macallum² are then added (1 c.c. if the bulk of the original precipitate was very small, otherwise 2 c.c.) and, correspondingly, 5 or 10 c.c. of 20 per cent. sodium carbonate solution are then added, and the colored solution is washed quantitatively into a 25 or 50 c.c. flask and diluted to the mark with distilled water. Compare the color of this solution, in a colorimeter, with that of one of the standards given below and calculate the results as there explained. In determining this point of dilution of the unknown solution, one compares roughly the color obtained at this point with that of the standard solution treated with the uric acid reagent and sodium carbonate solution. This standard solution should be prepared just prior to the addition of the car-

¹ This solution has the following composition: 70 c.c. of 3 per cent. silver lactate solution; 30 c.c. of magnesia mixture; and 100 c.c. of concentrated aqueous ammonia. The solution should be filtered before use. The magnesia mixture used in this solution is made as follows: Dissolve 17.5 grams of crystallized magnesium sulphate and 35 grams of ammonium chlorid in about 100 c.c. of water; add 60 c.c. of concentrated aqueous ammonia, and dilute to 200 c.c.

² The uric acid reagent is made as follows: To 750 c.c. of water add 100 grams of sodium tungstate and 80 c.c. of syrupy (86%) phosphoric acid. Boil gently for 2 hours, using a reflux condenser to prevent undue concentration. Cool the solution and dilute it to 1 liter in a volumetric flask. Two c.c. of this solution gives the maximum color obtainable with 1 mg. of uric acid. Benedict (Jour. Biol. Chem., 1922, LI, 187) now recommends the use of the following reagent, instead of the older one of Folin and Macallum. 100 grams of sodium tungstate ("Primos" brand if possible) and 30 grams of pure arsenic acid (As_2O_5) are placed in a liter flask and about 700 c.c. of water added. 50 c.c. of concentrated HCl are then added and the mixture is boiled for about 20 minutes, after which it is cooled and made up to 1 liter. Employ this reagent exactly as the previous one.

bonate to the unknown solution. Should the color of the unknown solution, when diluted to 25 c.c. be too deep for that of the standard solution, dilute to 50 c.c. in another volumetric flask, which should be at hand ready for this emergency. The same statement applies with reference to the dilution to 100 c.c. if necessary.

As Folin remarks, "No other detail in the colorimetric determination of uric acid has presented such difficulties as has the problem of finding a suitable standard." The first standard solution used, and one which gives accurate results but which is not at all permanent, is a solution of pure uric acid in lithium carbonate solution, prepared as follows: Two hundred and fifty mg. of Kahlbaum's pure uric acid are washed into a 250 c.c. volumetric flask by means of 25 to 50 c.c. of water. Add 25 c.c. of a 0.4 per cent. lithium carbonate solution, shaking at intervals for an hour before diluting. Make up to 250 c.c. in the flask and allow to stand for a few minutes before using. Each c.c. of this solution contains 1 mg. uric acid. In using this solution for comparison with the unknown solution of uric acid, take 1 c.c. of the standard solution, add 5 to 10 c.c. of water, 2 c.c. of the uric acid reagent and 20 c.c. of saturated sodium carbonate solution. Make up to the mark in a 100 c.c. volumetric flask. This solution is not advisable for the general laboratory as it necessitates the preparation of a fresh solution almost each time a uric acid determination is to be made and, hence, causes a needless loss of material owing to the fact that the solution does not keep longer than a few days.

Folin and Denis have, more recently, advised a permanent solution of uric acid, which meets the requirements in a general way but which must itself be standardized against the above solution of uric acid in lithium carbonate to obtain its colorimetric value. This solution is made by combining uric acid with formaldehyd as follows: One gram of uric acid is placed in a liter volumetric flask and dissolved by the addition of 200 c.c. of a 0.4 per cent. solution of lithium carbonate. To the solution are added 40 c.c. of 40 per cent. formaldehyd solution and the mixture is shaken and allowed to stand for a few minutes. Acidify the clear solution with 20 c.c. of normal acetic acid and dilute the mixture to the mark with water. The solution should remain perfectly clear and the next day (but not before) should be standardized against a freshly prepared lithium carbonate solution of uric acid as given above. The color produced by 5 c.c. of this solution corresponds very nearly to that obtained from 1 mg. of uric acid. The colorimetric reading obtained for this solution when compared against 1 mg. of pure uric acid, treated with the uric acid reagent and the sodium carbonate solution as above, is used as the standard value corresponding to 1 mg. of pure uric acid. It is seen, therefore, that this solution does not possess the reactivity corresponding to the actual amount of uric acid in it, so that its value must be determined against known uric acid solutions. This standardized permanent solution gives accurate figures and may be used as a routine.

Benedict and Hitchcock¹ have found a standard solution for use in this

¹ Jour. Biol. Chem., 1915, XX, 619. Curtman and Freed (Ibid., 1916, XXVIII, 89) advise the use of 25 c.c. of 4% boric acid solution, instead of the glacial acetic acid of Benedict and Hitchcock's standard, for use in the cold months. In warm weather this boric acid solution is inferior to the acetic acid solution.

test which seems to be an accurate and extremely permanent one. It has been used by the writer many times and is especially recommended for all comparisons in this test. The solution is as follows: Nine grams of pure crystallized disodium hydrogen phosphate, together with 1 gram of crystallized sodium dihydrogen phosphate are dissolved in 200 to 300 c.c. of hot water and the solution is filtered, if it was not perfectly clear. Make up the filtrate to a total of about 500 c.c. with hot water and pour this clear solution upon exactly 200 mg. of pure uric acid suspended in a few c.c. of water in a liter volumetric flask. Agitate the mixture for a few moments until the uric acid is completely dissolved and cool. Now add from a buret exactly 1.4 c.c. of glacial acetic acid and make up the contents of the flask to the liter mark with water. Mix the contents thoroughly and add about 5 c.c. of chloroform to prevent development of bacteria or moulds. Five c.c. of this solution contain exactly 1 mg. of uric acid. If there is any question of the standardizing of this solution, it may be checked up against standard solutions of uric acid in lithium carbonate once every month or so.

The calculation of the results obtained in the colorimetric determinations is not difficult. Treat the unknown solution and the standard solution with the prescribed amounts of uric acid reagent and sodium carbonate solution, diluting the standard (1 mg. of uric acid) to 100 c.c. and the unknown to 25, 50 or to 100 c.c. as stated above. Set the standard solution at 20 mm. and compare the colors. In making the calculation use the following formula:

$$\frac{20V}{RW} = \text{milligrams of uric acid per 100 c.c. of blood.}$$

In this formula 20 represents the standard reading, R gives the reading in mm. of the unknown solution, V represents the volume to which the unknown is diluted, and W represents the volume of blood taken for the determination.

Method of Folin and Wu.

This method¹ is included in the system of blood analysis advocated by these authors and may be followed in routine work, even though no other blood constituents are to be determined. In this method the uric acid is precipitated directly from the filtrate from the blood proteins.

Reagents Necessary.

1. A new standard uric acid solution. This is prepared as follows: Make up 1 to 3 liters of a 20 per cent. solution of sodium sulphite and let it stand over night. Filter the solution. Dissolve 1 gram of pure uric acid in 125 c.c. to 150 c.c. of 0.4 per cent. lithium carbonate solution and dilute to a volume of 500 c.c. Transfer 50 c.c. of this solution, corresponding to 100 mg. of uric acid to each of a series of volumetric liter flasks. Add 200 to 300 c.c. of water, then 500 c.c. of filtered 20 per cent. sodium sulphite solution, and finally make up to volume and mix well. Fill a series of 200 c.c. bottles and stopper very tightly with rubber stoppers. The solution in a bottle which is opened daily will keep for at least 3 to 4 months.

2. A 10 per cent. solution of sodium sulphite. This is prepared from the

¹ Jour. Biol. Chem., 1910, XXXVIII, 100. See Pucher, *Ibid.*, 1922, LII, 317 and 329.

excess of the 20 per cent. solution used in preparing the standard uric acid solutions. It may be kept in small tightly stoppered bottles.

3. A 5 per cent. solution of sodium cyanid, to be added from a buret when used in the test.

4. A 10 per cent. solution of sodium chlorid in 0.1N hydrochloric acid.

5. The uric acid reagent of Folin and Macallum, mentioned under the previous test.

6. A solution of 5 per cent. silver lactate in 5 per cent. lactic acid.

Technic.

To 10 c.c. of blood filtrate from the tungstic acid precipitation described under Non-protein Nitrogen in each of two centrifuge tubes add 2 c.c. of a 5 per cent. solution of silver lactate in 5 per cent. lactic acid, and stir with a fine glass rod. Centrifuge; add a drop of the silver lactate solution to the supernatant solution, which should be almost perfectly clear and should not become turbid when the last drop of silver solution is added. Remove the supernatant liquid by decantation as completely as possible. Add to each tube 1 c.c. of a solution of 10 per cent. sodium chlorid in 0.1 N hydrochloric acid and stir thoroughly with a glass rod. Then add 5 to 6 c.c. of water, stir again, and centrifuge once more. By this treatment the uric acid is set free from the precipitate. Transfer the two supernatant liquids by decantation to a 25 c.c. volumetric flask. Add 1 c.c. of a 10 per cent. sodium sulphite solution, 0.5 c.c. of a 5 per cent. solution of sodium cyanid,¹ and 3 c.c. of a 20 per cent. solution of sodium carbonate.

Prepare simultaneously two standard uric acid solutions as follows: Transfer to one 50 c.c. volumetric flask 1 c.c. and to another 50 c.c. flask 2 c.c. of the standard uric acid sulphite solution mentioned above. To the first flask add also 1 c.c. of 10 per cent. sodium sulphite solution. Then add to each flask 4 c.c. of the acidified sodium chlorid solution, 1 c.c. of the sodium cyanid solution, and 6 c.c. of the sodium carbonate solution. Dilute with water to about 45 c.c. When the two standard solutions and the unknown have been prepared, add 0.5 c.c. of the uric acid reagent to the unknown and 1 c.c. to each of the standards, and mix. Let stand for 10 minutes, fill to the mark with water, mix, and make the color comparisons.²

In connection with the calculation of the amount of uric acid in the unknown, it is to be noted that the blood filtrate taken (20 c.c.) corresponds to 2 c.c. of blood, that the standard is diluted to twice the volume of the unknown, and that the standard used contains 0.1 or 0.2 mg. of uric acid. The blood filtrate from blood containing 2.5 mg. of uric acid will be just equal in color to the weaker standard. Twenty times 2.5 divided by the reading of the unknown gives, therefore, the uric acid content of the blood when the

¹ Owing to the high toxicity of cyanid solutions, these should be measured only from burets, pipets never being employed.

² Oftentimes the color of the final solutions of the unknown is so faint that the color comparisons are made with great difficulties. Further, it is a not infrequent occurrence to have annoying turbidities appear in these final solutions, so that centrifugation or filtration must be employed to make it possible to make the color comparisons. Jackson and Palmer (Jour. Biol. Chem., 1922, L, 89) have modified this method in such a way that these turbidities are overcome.

weaker standard is set at 20 mm. Obviously, if the stronger standard is necessary and is set at 20 mm., the calculation is changed by the substitution of 5 for the 2.5 of the weaker standard. If a still weaker standard is needed, add 5 c.c. of 20 per cent. sodium carbonate solution to 25 c.c. of the regular weaker standard and dilute to 50 c.c. This latter corresponds to the color obtained from 1.25 mg. of uric acid per 100 c.c. of blood.¹

Benedict's Method with Folin and Wu Filtrates.

This method of Benedict² is much more simple than the preceding one and tends to give somewhat higher result than does the Folin and Wu procedure, at least on those bloods which contain less than 50 mg. of non-protein nitrogen. With bloods running 60 mg. or over of non-protein nitrogen, there is a much closer agreement with the methods.

The blood is precipitated with tungstic acid as described above by Folin and Wu, the blood being allowed to stand at least 10 to 20 minutes, after adding the tungstate and sulphuric acid, before filtration. This tends to insure complete protein precipitation. The use of excess of acid is to be avoided.

Five c.c. of the water-clear filtrate (representing 0.5 c.c. of blood) are transferred to a test-tube (these tubes need not be graduated but should be of uniform diameter, 18 to 20 mm.) and 5 c.c. of water are added. Five c.c. of the standard solution,³ containing 0.02 mg. of uric acid, are placed in another tube and the volume likewise made up to 10 c.c. To both standard and unknown are added 4 c.c. of 5 per cent. sodium cyanid solution (the addition always being made from a buret owing to the toxicity of the cyanid) containing 2 c.c. of concentrated ammonia per liter. To each tube is then added 1 c.c. of the arsenic-phosphoric acid-tungstic acid reagent.⁴ The contents of each tube should be mixed by one inversion immediately after addition of the reagent, and placed immediately in boiling water, where the tubes should be left for 3 minutes after immersion of the last tube, but the

¹ For other methods see Maase and Zondek, *Münch. med. Wchnschr.*, 1915, LXII 1105; Höst, *Ztschr. f. physiol. Chem.*, 1916, XCV, 88; Bogert, *Jour. Biol. Chem.*, 1917, XXXI, 165; Curtman and Lehrman, *Ibid.*, 1918, XXXVI, 157.

² *Jour. Biol. Chem.*, 1922, LI, 187. Morris and Macleod (*Ibid.*, 1922, L, 55 and 65), have introduced a method for uric acid employing as the reagent an arseno-tungstic acid.

³ Benedict states that it is advisable to keep on hand two standard solutions, one containing 0.01 mg. of uric acid per c.c., while the second contains 0.02 mg. of uric acid in 5 c.c. of solution. The second standard is the one commonly employed, but the first may occasionally be of service. To prepare the first solution, 25 c.c. of Benedict and Hitchcock's phosphate standard solution (see page 483) are measured into a 500 c.c. volumetric flask, and the flask filled about $\frac{3}{4}$ full with distilled water. 25 c.c. of dilute HCl (1 volume of concentrated HCl diluted to 10 volumes with water) are added, and the solution is diluted to 500 c.c. This solution contains 0.01 mg. uric acid in 1 c.c. For preparation of the second standard (the one which is most frequently employed) the procedure is the same, except that instead of starting with 25 c.c. of the phosphate solution, 10 c.c. are employed and diluted after acidification exactly as for the other standard. These standards should be freshly prepared once in 2 weeks.

⁴ 100 grams of pure tungstate are placed in a liter flask and dissolved in about 600 c.c. of water. 50 grams of pure arsenic pentoxid are now added, followed by 25 c.c. of 85 per cent. (syrupy) phosphoric acid and 20 c.c. of concentrated hydrochloric acid. The mixture is boiled for 20 minutes, cooled, and diluted to 1 liter. The reagent appears to keep indefinitely. This reagent yields nearly seven times as much color from a given weight of uric acid as does the old reagent of Folin and Macallum.

time elapsing between immersion of the first and last tubes should not exceed 1 minute. After the 3 to 4 minute heating, the tubes are removed and placed in a large beaker of cold water for 3 minutes and are read in the colorimeter as soon as may be convenient. Long standing before reading may lead to development of turbidity, so that it is advisable to read the solution within 5 minutes after removing from the cold water.

Calculation.—Employing the standard solution containing 0.20 mg. of uric acid and using 5 c.c. of the 1:10 blood filtrate, the calculation for the uric acid content of the original blood is as follows:

$$\frac{S}{R} \times 4 = \text{mg. uric acid per 100 c.c. of original blood.}$$

in which S represents the height of the standard solution and R the reading of the unknown. If, instead of using 5 c.c. of blood filtrate in the determination, 2.5 or 10 c.c. are employed, the final figure is multiplied or divided by 2 accordingly.

Amount of Uric Acid.

There is no question but that uric acid is present in appreciable amounts in normal blood, although such recognized investigators as Brugsch and Schittenhelm state that such is not the case. This divergence of opinion is due to the fact that the older methods were far from adequate. In one of their reports, Folin and Denis give the normal uric acid values of human blood as varying from 1 to 2.5 mg. per 100 grams of blood. Concerning these figures they state that "we are not prepared to say that they represent the full variations." A rise in the uric acid values undoubtedly occurs following a meal rich in nuclein-containing food, but the extent of this "digestive rise" has not been definitely established. Weintraud has reported the presence of 5 mg. of uric acid per 100 c.c. of blood during digestion. This must point to the fact that the kidneys have a limited power of excreting uric acid when a surplus is suddenly poured into the blood from the digestive tract. Sooner or later, however, this digestive accumulation is adjusted and the blood findings become more nearly the figures given above as normal. Denis¹ has shown that in normal men no increase in the circulating uric acid is produced by the ingestion of large quantities of purins. In persons suffering from renal insufficiency a more or less marked increase in the uric acid content of the blood is produced by high purin feeding. He concludes, therefore, that when the determination of uric acid in the blood is undertaken as a diagnostic test, the insistence on a preliminary period during which no purin-containing foods are consumed is unnecessary except where a renal insufficiency exists. It would thus seem that the normal kidney reacts toward an excess of uric acid in a way essentially similar to that in which it conducts itself toward an excess of urea and is able to excrete the excess of uric acid, thereby keeping the circulating uric acid at the same level as that obtained when only endogenous uric acid is to be excreted. While Folin and Denis have reported increased amounts of uric acid in pathological conditions, especially in nephritis, it is interesting to note "that there is

¹ Jour. Biol. Chem., 1915, XXIII, 147.

apparently no relationship between the amount of uric acid and the amount of urea or total non-protein nitrogen in human blood." While the urea and total non-protein nitrogen of the blood are inversely proportional to the general efficiency of the kidney, the significance of the uric acid is less clear. According to Folin, we may have four distinct classes of blood with reference to uric acid: (1) blood in which both uric acid and non-protein nitrogen are present in normal amounts; (2) blood in which with normal amount of uric acid we have greatly increased amount of non-protein nitrogen; (3) blood giving abnormally high uric acid values with normal amounts of non-protein nitrogen; and (4) blood in which abnormally large amounts of both uric acid and non-protein nitrogen are present.

The condition of increase of uric acid in the blood is known as uricacidemia or lithemia. In cases of severe nephritis, in which the eliminatory power of the kidney is greatly reduced, a relatively marked increase in the uric acid of the blood is observed. This increase in nephritis reaches the highest values for the uric acid of the blood, being as high as 10 mg. in some cases, and is associated with high non-protein nitrogen and urea values. As Myers and Fine¹ state, the kidney normally concentrates the creatinin 100 times, the urea 80 times, but the uric acid only 20 times. It would seem, therefore, that high uric acid values would be shown much earlier than those of creatinin and urea which fact should be of value in early diagnosis. Chace and Myers² report uric acid values as high as 22.4 mg. per 100 c.c. of blood in cases of fatal chronic interstitial nephritis. An increase in the uric acid is, also, observed in all conditions associated with increased nuclein decomposition. In this group we find leukemia, pneumonia, carcinoma and severe febrile conditions. Further, an increase is noted in practically all cases of chronic lead poisoning, due, perhaps, to the associated renal degeneration.

For some time following the teaching of Garrod, an accumulation of uric acid in the blood was held to be pathognomonic of gout. That this idea is erroneous is seen from the list of conditions in which an increase is observed. In fact these other diseases frequently show a uric acid value much higher than is ever reached in gout. The usual increase in gout runs from 3 to 7 mg. According to Magnus-Levy, there is no increase of uric acid in the blood preceding or during the gouty attack, but a marked accumulation follows the seizure. Folin's findings would seem to indicate that in gout there is almost invariably an abnormally high uric acid value while the total non-protein nitrogen is within normal limits. In non-gouty arthritis the blood is not infrequently high in uric acid, but most cases have abnormally high non-protein nitrogen. Hence, for differential diagnosis in a case of suspected gout, the uric acid value is of no importance in itself, but must be taken in connection with a high or low non-protein nitrogen or urea.³

¹ Jour. Biol. Chem., 1919, XXXVII, 239.

² Jour. A. M. A., 1916, LXVII, 929; see, also, Myers, Fine, and Lough, Arch. Int. Med., 1916, XVII, 570.

³ See Folin and Macallum, Jour. Biol. Chem., 1912, XIII, 363; Folin and Denis, Ibid., 469; Ibid., 1913, XIV, 29 and 95; Brugsch and Kristeller, Deutsch. med. Wchnschr., 1914, XL, 746; Weiss, New York Med. Jour., 1914, C, 180; Adler and Ragle, Boston Med. and

Ammonia.

Normally the blood contains ammonium compounds to a very slight extent, the limits not having yet been determined. This ammonia arises partly from the normal pancreatic digestion and finds its way into the circulation as a precursor of urea. Moreover, it is especially absorbed into the blood as a product of bacterial putrefactive processes in the large intestine, as Folin and Denis have shown. As a further source one finds the metabolic activities so adjusted that any excess of acids, introduced into the system from without or produced within the system by increased protein decomposition, is neutralized up to a certain point by a corresponding increase in the ammonia produced. It is, in this case, to be regarded as a stage in the deamidization of the proteins, being formed when certain nitrogenous groups are liberated from the amino acids, which are linked together to form the protein molecule. We are justified, therefore, in assuming that a preceding increase of ammonium compounds in the blood must obtain, to account for an excretion of such an excess of ammonia as we find in the urine under certain conditions already discussed. However, Nash and Benedict¹ have offered contradictory evidence. They found no noteworthy increase in the ammonia content of the blood in the general circulation, even in conditions in which the urinary output of ammonia was markedly augmented; nor was there any accumulation when the kidney function was seriously interfered with, although the content of other nitrogenous excretory products was decidedly increased. As these workers found twice as much ammonia in the blood from the renal vein as from other sources, they conclude that the kidney, instead of excreting ammonia from the blood, forms the ammonia which it excretes, while at the same time it contributes a small amount of ammonia to the blood. It is probably true that the actual amount of ammonium compounds in the systemic blood is almost infinitesimal under normal conditions, although the portal blood is rich in such elements. It is evident, therefore, that what ammonia does find its way into the blood is, to a considerable extent, rapidly removed therefrom, probably through the action of the ammonia-destroying function of the liver. Whether an hepatic insufficiency may be demonstrated

Surg. Jour., 1914, CLXXI, 769; Krocher, Deutsch. Arch. f. klin. Med., 1914, CXV, 380; Steinitz, Deutsch. med. Wchnschr., 1914, XL, 953; Fine and Chace, Jour. Pharmacol. and Exper. Therap., 1914, VI, 219; Liefmann, Ztschr. f. Kinderhkte., 1915, XII, 227; Benedict and Hitchcock, Jour. Biol. Chem., 1915, XX, 619; Benedict, *Ibid.*, 629 and 633; Folin and Denis, Arch. Int. Med., 1915, XVI, 33; Masse and Zondek, Münch. med. Wchnschr., 1915, LXII, 1105; Höst, Norsk Mag. f. Laegevidensk., 1915, LXXVI, 1112; Denis (Jour. Pharmacol. and Exper. Therap., 1915, VII, 255) shows the increased elimination of uric acid following use of salicylates; Jörgensen, Ugeskr. f. Laeger, 1915, LXXVII, 1429; Fine, Jour. A. M. A., 1916, LXVI, 2051; Pratt, Am. Jour. Med. Sc., 1916, CLI, 92; N. Y. State Jour. Med., 1916 XVI, 531; Fine, *Ibid.*, 541; Watanabe, Am. Jour. Med. Sc., 1917, CLIV, 76; Kingsbury and Sedgwick, Jour. Biol. Chem., 1917, XXXI, 261; Slemmons and Bogert, *Ibid.*, XXXII, 63; Baumann, Hausmann, Davis, and Stevens, Arch. Int. Med., 1919, XXIV, 70; Upham and Higley, *Ibid.*, 557; Chauffard, Brodin, and Grigaut, Presse Med. 1920, XXVIII, 905; Myers, Jour. Lab. & Clin. Med., 1920, V, 490; Theis and Benedict, *Ibid.*, 1921, VI, 680; Williams, Jour. Am. Med. Assoc., 1921, LXXVI, 1207; Chauffard, Sigle Med., 1921, LXVIII, 675 and 700; Krauss, Deutsch. Arch. f. klin. Med., 1922, CXXXVIII, 340; Grigaut, Bull. soc. chim. biol., 1922, IV, II; Brugsch, Med. Klin., 1922, XVIII, 553; Bauman and Keeler, Jour. Lab. and Clin. Med., 1922, VII, 551.

¹ Jour. Biol. Chem., 1921, XLVIII, 463; *Ibid.*, 1922, LI, 183.

by later researches on the ammonia of the blood is a matter of speculation. Rohde, in her excellent studies with the vividiffusion apparatus of Abel, has shown that there is no liberation of ammonia comparable to that which takes place under aseptic conditions in shed blood, and further, that no ammonia is formed from the diffusible constituents of the blood.

The amount of ammonia in the circulating blood has, as mentioned above, not been definitely determined. The figures given by different workers are somewhat at variance, due, no doubt, to the fact that decomposition of nitrogenous compounds begins as soon as the blood is drawn and, in consequence, the amount of ammonia may increase by leaps and bounds in a few hours. Hence, it is absolutely essential, in the use of any method for the determination of ammonia in the blood that the work be done rapidly. In his earlier studies of the ammonia of the blood, Folin showed that the ammonia nitrogen of the blood from the mesenteric vein of the large intestine of cats ranged from 0.24 to 1.6 mg. per 100 c.c. of blood; while that from the portal vein of the same animals showed a range of a trace only to 0.22 mg. per 100 c.c.; and that from the small intestine ranges from 0.05 to 0.77 mg. per 100 c.c. It is, probably, true that the upper limit of the ammonia nitrogen of human blood is 1 mg. per 100 c.c. Thus, Gettler and Baker, using the method of Folin and Denis, find an average of between 0.4 and 0.75 mg.; Barnett, with a somewhat modified technic, finds an average in five normal men of between 0.07 and 0.1 mg. Folin and Denis find less than 1 mg. and Folin states that when precautions are taken to render the potassium oxalate and permittit, used in the method of Morgulis and Jahr, ammonia-free "the amount of ammonia obtained from protein-free blood filtrates is practically nothing and only a greenish yellow color is obtained on Nesslerizing." Morgulis and Jahr find the norma values, by their method to range from 0.14 to 0.30 mg per 100 c.c. of blood.

While the normal ammonia is relatively insignificant in amount, yet quite an appreciable amount is shown by several workers in pathologic conditions. While, as stated above, ammonia is rapidly formed when blood stands after being drawn, yet the increase noted in certain cases can hardly be assumed to be entirely due to this factor. McNeil and Levy state that "In no instances, however, have we found the ammonia content of sterile normal blood to rise above 3 mg. per 100 c.c. even after prolonged standing, nor have any of the observers mentioned above found the rise in ammonia to exceed this limit after standing." Hence they consider that under any circumstances a finding of over 3 mg. per 100 c.c. may be safely taken as abnormal. In a recent contribution by Gad-Andersen, it is shown that the ammonia nitrogen of muscle tissue varies from a value of 0.8 mg. per 100 c.c. of blood (the determination being made at once) to 5.0 mg. per 100 c.c. 2 hours later. He believes that the concentration of ammonia is the same in muscle and blood and that the increase in ammonia, shown on allowing the material to stand before determining the ammonia, is due to the decomposition of the urea of the muscle. This would seem to refute the statements of Sumner as well as those of Marshall and Davis, although these latter workers

conclude that the concentration of urea in blood and tissues is approximately the same even though they did not find the relatively large amounts of ammonia reported by Gad-Andersen. McNeil and Levy have reported an extensive study of the blood ammonia in several series of cases with the following results: In chronic diseases of the liver, a range of ammonia from a trace to 6.8 mg. per 100 c.c.; in myocarditis, a trace to 17 mg. per 100 c.c.; in acidosis from various causes, a trace to 7 mg. per 100 c.c.; in nephritis, a trace to 9 mg. per 100 c.c. of blood.

Owing to the small amount of ammonia present in the blood and its rapid formation from other substances on standing, the methods advanced for its determination are not entirely satisfactory. The recent technic of Morgulis and Jahr would appear to be very satisfactory, were it not that certain amino acid derivatives present in the blood filtrate are absorbed by the permutit used and introduce a variable factor in the Nesslerization process. For this reason we advise the method of Folin and Denis, which is much older and gives good comparative figures, although it has been shown by Henriques and Christiansen that the amount of ammonia recovered may vary with the rapidity of the aeration and the temperature. The method of Barnett is cumbersome and requires the most assiduous efforts to prevent variations in the titer of the solutions used in the micro-titration of the ammonia. As such determinations do not, at present, offer any clinical advantages, the writer is content with reference to the literature.¹

Creatinin.

The number of creatinin and creatin determinations in blood recorded in the literature is very small. Only within the last few years has the importance of a study of the variations in the creatinin content of the blood been recognized. Folin and Denis report that they have found no specific creatinin retention, the figures in some 200 cases indicating that the human kidneys remove creatinin from the blood with remarkable ease. The normal creatinin content of the blood appears to lie between 1 and 2 mg. per 100 c.c. of blood, while the creatin value runs as high as 5 or 8 mg. It is, no doubt, true that a retention of creatinin occurs, practically speaking, only in nephritis. In such cases we find the creatinin values paralleling those of non-protein nitrogen and urea, high retention being observed only in the severe cases which show a tendency toward uremia. Myers and Fine report as high creatinin values as 33 mg. per 100 c.c. of blood in cases of this type. Myers and Lough believe the estimation of blood creatinin to be of considerable prognostic value, figures from 2.5 to 3 mg. being suspicious, those from 3 to

¹ Folin, *Ztschr. f. physiol. Chem.*, 1902, XXXVII, 161; Medwedew, *Ibid.*, 1911, LXXII, 410; Folin and Denis, *Jour. Biol. Chem.*, 1912, XI, 161 and 527; Taylor and Ringer, *Ibid.*, 1913, XIV, 407; Jacobson, *Ibid.*, 1914, XVIII, 133; Fiske and Karsner, *Ibid.*, 381; Rohde, *Ibid.*, 1915, XXI, 325; Henriques and Christiansen, *Biochem. Ztschr.*, 1916, LXXVIII, 105; Barnett, *Jour. Biol. Chem.*, 1917, XXIX, 459; Barnett and Addis, *Ibid.*, XXX, 41; McNeil and Levy, *Jour. Lab. and Clin. med.*, 1917, II, 509; *Ibid.*, III, 18; Morgulis and Jahr, *Jour. Biol. Chem.*, 1919, XXXVIII, 435; Folin, *Ibid.*, XXXIX, 250; Gad-Andersen, *Ibid.*, 207; Gerard, *C. R. soc. de biol.*, 1910, p. 1180; Nash and Benedict, *Jour. Biol. Chim.*, 1921, XLVIII, 463; Gad-Andersen, *Ibid.*, 1922, LI, 307; Strauss, *Zentralbl. f. inn. Med.*, 1922, XLIII, 25.

5 mg. being regarded as decidedly unfavorable, while values over 5 mg. probably indicate an early fatal termination. Veeder and Johnston show, from a study of 75 cases of normal children as well as those with scarlet fever and other clinical conditions, that the normal values of creatinin range from 0.58 to 3.44 mg., while the range in febrile conditions is from 1.08 to 3.82 mg.; the normal creatin is rarely over 5 mg. with no specific relation obtaining between creatinin and creatin. The values reported by Gettler and Baker for creatinin are remarkably low, being 0.1 to 0.5 mg.; while creatin shows a range of 3.0 to 6.5 mg. Since the introduction of the Folin picric acid method for the determination of creatinin, it has been shown by several workers that the results by this method were somewhat higher than the true values. Folin and Doisy, Wilson and Plass, and Hunter and Campbell have shown that picric acid gives high values, due to the fact that the acid is impure so that erroneous colorations arise when the solutions are treated with alkalies. Hunter and Campbell, working on the distribution of creatinin and creatin between the plasma and corpuscles find the following: Creatinin of normal human blood plasma ranges between 0.7 and 1.3 mg. per 100 c.c., it being practically certain that this is distributed through the corpuscles and plasma in uniform concentration; creatin of the blood is chiefly concentrated in the corpuscles, the range being 6 to 9 mg. per 100 c.c., while the plasma shows not more than 0.4 to 0.6 mg. per 100 c.c.; the values of creatin in the whole blood average about 3 mg. per 100 c.c. Greenwald and McGuire give as the normal values, 3 to 5 mg. per 100 c.c. of total creatinin, with a normal creatin value of 1.4 to 3.7 mg. per 100 c.c. They regard anything over 5 mg. of total creatinin as suspicious, 6 as pathological, and 10 very serious. Denis, using his proposed new method, finds the total creatinin of human blood ranging from 4 to 8 mg. per 100 c.c. in a series of pathological cases. Behre and Benedict seem to be convinced that creatinin does not exist in detectable quantities in the blood, but that creatin is constantly present in appreciable amounts. It is evident, therefore, that the earlier figures, as reported for creatinin and creatin, must be regarded as high due to the errors in the methods used. By the use of the new method of Folin and Wu (outlined below) the total creatinin value for normal blood is given as 6 mg. per 100 c.c., which is quite at variance to that of the earlier method of Folin.¹ From the distribution of the non-protein nitrogenous compounds in the blood and urine, it would appear that of the three constituents usually estimated creatinin is normally the most readily, and uric acid the

¹ Folin and Denis, *Jour. Biol. Chem.*, 1912, XII, 141; *Ibid.*, 1914, XVII, 475, 487, and 493; Neubauer, *Münch. Med. Wehnschr.*, 1914, LXI, 857; Myers and Fine, *Jour. Biol. Chem.*, 1915, XX, 391; Foster, *Arch. Int. Med.*, 1915, XV, 350; Myers and Lough, *Ibid.*, XVI, 536; Woods, *Ibid.*, 577; Veeder and Johnston, *Am. Jour. Dis. Child.*, 1916, XII, 136; Gettler and Baker, *Jour. Biol. Chem.*, 1916, XXV, 214; McCrudden and Sargent, *Ibid.*, XXVI, 527; Plass, *Bull. Johns Hopk. Hosp.*, 1917, XXVIII, 137; Folin and Doisy, *Jour. Biol. Chem.*, 1917, XXVIII, 349; Gettler, *Ibid.*, XXIX, 47; Wilson and Plass, *Ibid.*, 413; Hunter and Campbell, *Ibid.*, XXXII, 195; *Ibid.*, 1918, XXXIII, 169; Greenwald and McGuire, *Ibid.*, XXXIV, 193; Denis, *Ibid.*, XXV, 513; Myers and Killian, *Proc. Soc. Exp. Biol. and Med.*, 1918, XVI, 41; Watanabe, *Kyoto Igaku Zasshi*, 1918, XV, 72; Meyers and Killian, *Am. Jour. Med. Sc.*, 1919, CLVII, 674; Feigl, *Biochem. Ztschr.*, 1920, CV, 255; Wang and Dentler, *Jour. Biol. Chem.*, 1920, XLV, 237; Rabinowitch, *Can. Med. Assoc. Jour.*, 1921, XI, 320; Behre and Benedict, *Jour. Biol. Chem.*, 1922, LII, 11.

least readily, eliminated, with urea standing in an intermediate position. It seems reasonable to conclude that when a noticeable retention of creatinin has occurred, the functional condition of the kidney has been markedly impaired.

Method of Folin and Wu.

This method¹ was introduced in order to avoid the errors incident to the determination of creatin when picric acid was used as a protein precipitant. While, perhaps, no more accurate than the methods of Greenwald and McGuire or of Denis, yet it is more convenient than these as it forms part of the system of blood analysis, which permits the determination of practically all the non-protein nitrogenous constituents with one precipitation of protein.

Determination of Preformed Creatinin.

Transfer 25 (or 50) c.c. of a saturated solution of picric acid (purified by the method of Folin and Doisy, see urine page 269) to a small clean flask, add 5 (or 10) c.c. of 10 per cent. sodium hydrate solution and mix. Transfer 10 c.c. of the tungstic acid blood filtrate (discussed under non-protein nitrogen) to a small flask or to a test tube. Transfer 5 c.c. of the standard creatinin solution² to another flask and dilute this to 20 c.c. Then add 5 c.c. of the alkaline picrate solution, freshly prepared as above, to the blood filtrate, and 10 c.c. to the diluted standard creatinin solution. Let stand for 8 to 10 minutes and make the color comparison in the usual manner, never omitting first to ascertain that the two fields of the colorimeter are equal when both cups contain the standard creatinin picrate solution. This color comparison should be completed within 15 minutes from the time the alkaline picrate was added.

When the amount of blood filtrate available for the creatinin determination is too small to permit repetition, it is of course advantageous or necessary to start with more than one standard. If a high creatinin should be encountered unexpectedly without several standards ready, the determination may be saved by diluting the unknown with an appropriate amount of the alkaline picrate solution—using for such dilution a picrate solution first diluted with two volumes of water—so as to preserve equality between the standard and the unknown in relation to the concentration of picric acid and sodium hydrate.

Calculation.—The reading of the standard in mm. (usually 20) multiplied by 1.5, 3, 4.5, or 6 (according to how much of the standard solution was

¹ Jour. Biol. Chem., 1919, XXXVIII, 98.

² One standard creatinin solution, suitable both for creatinin and creatin determinations in blood, can be made as follows: Transfer to a liter flask 6 c.c. of the standard creatinin solution described on page 271 (which contains 6 mg. of creatinin); and 10 c.c. of normal hydrochloric acid, dilute to the mark with water, and mix. Transfer to a bottle and add 4 or 5 drops of toluene or xylene. Five c.c. of this solution contains 0.03 mg. of creatinin, and this amount plus 15 c.c. of water represents the standard needed for the vast majority of human blood, for it covers the range of 1 to 2 mg. per 100 c.c. In the case of unusual bloods representing retention of creatinin, take 10 c.c. of the standard plus 10 c.c. of water—which covers the range of 2 to 4 mg. of creatinin per 100 c.c. of blood; or 15 c.c. of the standard plus 5 c.c. of water, by which 4 to 6 mg. may be estimated. By taking the full 20 c.c. volume from the standard solution at least 8 mg. may be estimated; but when working with such blood, it is probably advisable to substitute 5 c.c. of blood filtrate plus 5 c.c. of water for the usual 10 c.c. of blood filtrate taken for the test.

taken), and divided by the reading of the unknown in mm., gives the amount of creatinin in mg. per 100 c.c. of blood. In connection with this calculation it is to be noted that the standard is made up to twice the volume of the unknown, so that each 5 c.c. of the standard creatinin solution, while containing 0.03 mg., corresponds to 0.015 mg. in the blood filtrate.

Determination of Creatin plus Creatinin.

Transfer 5 c.c. of the tungstic acid blood filtrate to a test-tube graduated at 25 c.c. Add 1 c.c. of normal hydrochloric acid. Cover the mouth of the test-tube with tin-foil and heat in the autoclave to 130°C . for 20 minutes, or to 155°C . for 10 minutes. Cool. Add 5 c.c. of the alkaline picrate solution and let stand for 8 to 10 minutes, then dilute to 25 c.c. The standard solution required is 20 c.c. of creatinin solution in a 50 c.c. volumetric flask. Add 2 c.c. of normal hydrochloric acid and 10 c.c. of the alkaline picrate solution, and, after 10 minutes standing, dilute to 50 c.c. The preparation of the standard must, of course, have been made first so that it is ready for use when the unknown is ready for the color comparison. The height of the standard, usually 20 mm., divided by the reading of the unknown and multiplied by 6 gives the "total creatinin" in mg. per 100 c.c. of blood. In the case of uremic bloods containing large amounts of creatinin, 1, 2, or 3 c.c. of blood filtrate, plus water enough to make approximately 5 c.c. are substitutes for 5 c.c. of the undiluted blood filtrate. The normal value for "total creatinin" by this method is about 6 mg. per 100 c.c. of blood.

Amino Acids.

Much discussion has, for long years, centered around the occurrence of amino acids in the blood. It is well known that these nitrogenous bodies, both of the mono- and di-amino types, are formed in the intestinal digestion of protein material. As the methods of research were for the most part inadequate for the detection of these bodies, it was assumed that the blood did not contain them but that they were either resynthesized in passing through the absorptive process or were at once converted by the liver into urea. Both of these ideas have been shown to be fallacious, thanks to the work of Folin, Van Slyke and Meyer, Abderhalden (who was formerly one of the staunchest opponents of the presence of these bodies in the blood), and more recently of Abel, Rowntree and Turner. Many of these amino acids have been isolated from the blood and identified by very careful chemical examination, so that there is no longer any doubt of their occurrence in the blood stream. Whether or not variations in the amounts of these bodies, leading to a hyper-amino-acidemia or to a hypo-amino-acidemia will prove to be of clinical value remains to be seen. Ellis, Cullen and Van Slyke report the amino-acid nitrogen of the blood as varying within certain definite limits (4.5 to 8.5 mg. per 100 c.c.) in different individuals and in the same individual at different times. Schlutz and Pettibone, in their studies of children, show that the average is 4 mg. per 100 c.c. of blood in normal cases, while in pathological conditions the range is from 1.22 to 6.97 mg., cases of nephritis showing only 1.24 to 4.44 mg. Bock, using a slightly modified Greenwald method, finds the average normal

adult figures to be 7.13 mg. In pathological cases, the range is from 4.5 to 30 mg. per 100 c.c., the most pronounced increase being noted in nephritis.¹

Folin's Method.²

Transfer to a test-tube (capacity 30 to 35 c.c.) 1 c.c. of the standard acid glycocoll (glycine) solution representing 0.07 mg. of nitrogen³ and add 3 c.c. of water. To another similar test-tube add 5 c.c. of the blood filtrate obtained as discussed on page 497. Add 1 drop of 0.25 per cent. phenolphthalein solution to each. Add 1 c.c. of the 1 per cent. sodium carbonate solution⁴ to the standard and then add carefully, drop by drop, enough of the sodium carbonate solution to the blood filtrate until it has approximately the same pink color as the standard (3 or 4 drops are usually required). Add another 5 c.c. of water to the standard, as the volume of the standard is to be twice that of the blood filtrate. Then prepare a fresh 0.5 per cent. solution of the sodium salt of β -naphthoquinone-sulphonic acid;⁵ add 2 c.c. of this solution to the standard and 1 c.c. to the blood filtrate. Shake a little to make the solutions uniform and set them aside in a completely dark cupboard and leave them there till the following day; that is, for 19 to 30 hours. At the end of the time specified, add the acetic acid-acetate solution⁶—2 c.c. to the standard and 1 c.c. to the blood filtrate. After the acetic acid has been added (never before) add the thiosulphate solution⁷—2 c.c. to the standard and 1 c.c. to the blood filtrate. Finally add with a "blood pipet" 14 c.c. of water to the standard giving a volume of 30 c.c. and add 7 c.c. of water to the blood filtrate giving a final volume of 15 c.c. Mix and make the color comparison, setting the standard at 20 mm. Twenty divided by the colorimetric reading of the unknown, times 7; or 140 divided by the colori-

¹ In this connection see Folin, *Jour. Biol. Chem.*, 1912, XI, 87; Van Slyke, *Ibid.*, 1911, IX, 185; *Ibid.*, 1912, XII, 275; *Ibid.*, 1913, XVI, 121 and 125; Van Slyke and Meyer, *Ibid.*, 1912, XII, 399; Abderhalden, *Ztschr. f. physiol. Chem.*, 1913, LXXXVIII, 478; Kaplan, *New York Med. Jour.*, 1913, XCVII, 1172; *Ibid.*, 1913, XCVIII, 157; Kaplan and McClelland, *Ibid.*, 1912; Abel, Rowntree and Turner, *Jour. Pharmacol. and Exper. Therap.*, 1914, V, 611; Ellis, Cullen and Van Slyke, *Jour. Am. Med. Assn.*, 1915, LXIV, 126; György and Zunn, *Jour. Biol. Chem.*, 1915, XXI, 511; Schlutz and Pettibone, *Am. Jour. Dis. Child.*, 1915, X, 206; Pettibone and Schlutz, *Jour. A. M. A.*, 1916, LXVII, 202; Ross, *Jour. Biol. Chem.*, 1916, XXVII, 45; Bock, *Ibid.*, 1917, XXVIII, 357; *Ibid.*, XXIX, 191; Okada, *Ibid.*, 1918, XXXIII, 325; Schweriner, *Ztschr. f. exp. Pathu. Ther.*, 1920, XXI, 129; Kozana and Mayprints, *Biochem. Jour.*, 1921, XV, 167; Okada and Hayashi, *Jour. Biol. Chem.*, 1922, LI, 121; Folin and Berglund, *Ibid.*, 395.

² *Jour. Biol. Chem.*, 1922, LI, 377.

³ The standard stock solution is made by dissolving 53.5 mg. of glycocoll in 100 c.c. of 0.1 N hydrochloric acid containing 0.2 per cent. of sodium benzoate. From the stock solution, containing 0.1 mg. of nitrogen per c.c., the blood standard is made by diluting 70 c.c. with 0.1 N hydrochloric acid to a volume of 100 c.c.

⁴ Fifty c.c. of approximately saturated solution (22 per cent.) of sodium carbonate are diluted to a volume of 500 c.c. The strength of the resulting solution is determined by titrating 20 c.c. of 0.1 N HCl with the carbonate, using methyl red as indicator. On the basis of the titration value thus obtained, the carbonate solution is diluted so that 8.5 c.c. are equivalent to 20 c.c. of the 0.1 N acid. This is about a 1 per cent. solution.

⁵ Folin gives the method for making this quinone in pure form. As solutions of it become dark in a short time, only fresh solutions must be used for the color work. Transfer 100 mg. of the quinone to a small flask, add 20 c.c. of water, and shake. One c.c. of this solution is used for 5 c.c. of blood filtrate.

⁶ Dilute 100 c.c. of 50 per cent. acetic acid with an equal volume of 5 per cent. sodium acetate solution.

⁷ A 4 per cent. solution of sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$). This is used to destroy the surplus quinone remaining after the full color obtainable from the amino-acids has developed.

metric reading gives the amino-acid nitrogen in milligrams per 100 c.c. of blood.

(E) Carbohydrates.

The presence of sugar in the blood, both of normal and of abnormal types, is a well demonstrated fact. Whether this sugar is in the free molecular state and is dialyzable or whether it is combined and non-dialyzable is still a matter of discussion. The discovery of jecorin (a combination of glucose with lecithin) by Drechsel indicates that such combinations occur in the blood, but it has by no means been proven that such compounds are the usual blood sugar. The work of Michaelis and Rona, Tachau, Gradwohl and Blaivas, along with others would seem to indicate that the sugar is in the free state; Lepine and Boulud, Kleiner, and others believe that the sugar is in combination. It is an interesting finding of Gradwohl and Blaivas that the amount of sugar is practically the same in the whole blood, plasma, and cells. Lepine states that certain blood samples may contain both free and combined sugar, both of which may be shown to dialyze. Michaelis and Rona point out that if both free and combined sugar exist side by side in the blood they are probably in equilibrium with one another; when the blood is dialyzed the free sugar will pass out thus destroying the equilibrium and forcing the decomposition of a part of the combined sugar. While other substances, such as uric acid and creatinin, are present in the blood and show a reducing action, yet there can be no question of the presence of true sugar itself. The sugar usually present is glucose, but maltose is occasionally found in the blood of nursing mothers, while levulose and pentose may occur in the blood and urine after intake of large amounts of food containing them.¹

Glucose.

The occurrence of glucose in the blood is termed glycemia. This hexose is found normally in quantities varying from 0.4 to 1.5 parts per 1000, the average being probably about 0.8 per 1000 or, in other words, 0.08 gm. per 100 c.c. of blood. Under pathological conditions this amount may increase to as high as 1.1 gm. per 100 c.c., as reported by Myers and Bailey. According to Claude Bernard, whenever the sugar of the blood reaches three parts per 1000, diuresis and glycosuria occur as evidences of the systemic effort to control the hyperglycemia. This figure is undoubtedly much too high and was obtained with methods which could hardly compare with those of the present day. The study of the "renal threshold," by which is meant the height of the blood sugar level at which appreciable amounts of sugar are eliminated in the urine, by more exact methods shows that, when the blood sugar reaches 0.15 gm. per 100 c.c., sugar will appear in the urine, although Hamman and Hirschman place this value between 0.17 and 0.18 gm. It is

¹ Michaelis and Rona, *Biochem. Ztschr.*, 1900, XVI, 60; Lepine and Boulud, *Lyon Méd.*, 1913, XLV, 997; von Hess and McGuigan, *Jour. Pharmacol. and Exp. Therap.*, 1914, VI, 45; Tachau, *Ztschr. f. klin. Med.*, 1914, LXXIX, 421; Canavan and Dahlstrom, *Wisc. Med. Jour.*, 1916, XV, 151; Lepine, *Jour. physiol. et path. gen.*, 1917, XVII, 377; Gradwohl and Blaivas, *Jour. Lab. and Clin. Med.*, 1917, II, 416; Kleiner, *Jour. Biol. Chem.*, 1918, XXXIV, 471.

important to remember, in this connection, that this value is not to be regarded as a constant, as many normal individuals may excrete sugar with a blood sugar value of 0.14 gm. or even less; while others may show even higher values than 0.18 gm. The usual test of the "tolerance" for sugar, of administering 100 grams of glucose on an empty stomach and studying its effect both on the sugar of the blood and urine, indicates that only a moderate hyperglycemia results and that this rapidly subsides, the blood sugar reaching normal values in about 2 hours. With a diabetic, however, we find that a lowered tolerance usually exists and that the hyperglycemia may reach more than 0.2 gm., the reaction lasting for 3 to 5 hours. Williams and Humphrey point out that there is no striking relation between the height of the renal threshold and the duration of the diabetes, although it would appear that the threshold tends to rise with the increasing duration of the disease. When the diabetes is mild or quiescent, the point at which the kidneys eliminate sugar is stationary; but when the disease becomes progressive, the threshold tends to rise, a rising threshold in the face of careful dietary treatment, being regarded as a serious prognostic sign. It will be seen, therefore, that a study of the "renal threshold" will, in a measure at least, enable us to differentiate between a true diabetes and a so-called "renal diabetes," although it is probable that no definite level can be fixed where a true diabetes begins owing to the variability of the normal value. Certain it is, however, that values about 0.17 should be regarded with great suspicion.

While high values for blood sugar are observed in diabetes, we find, also, in nephritis that an increase, often above 0.2 gm. per 100 c.c. obtains. Many nephritics have a high renal threshold, while others, of apparently the same clinical type, have a normal threshold. The literature contains many studies of the blood sugar in nephritis and the general trend of the reports is toward an increase in the blood sugar. These values have, for the most part, been obtained by the methods of Lewis and Benedict, or the Myers and Bailey modification of this method, so that it is probable that the results are higher than those really obtaining, owing to the fact that creatinin, which is increased to an appreciable extent, reacts with the picric acid used in the test and tends to give false values for the blood sugar.¹ While Benedict does

¹ Reicher and Stein, *Ztschr. f. exper. Path. u. Therap.*, 1912, X, 532; Herzfeld, *Ztschr. f. physiol. Chem.*, 1912, LXXVII, 420; Rolly and Oppermann, *Biochem. Ztschr.*, 1913, XLVIII, 50, 187, 200, 259, 268 and 471; *Ibid.*, 1913, XLIX, 278; Jacobsen, *Ibid.*, 1913, LVI, 471, 472; and Strassner, *Med. Klin.*, 1913, IX, 1462; Bing and Jakobsen, *Ugeskr. f. Læger*, 1913, LXXXV, 1627; Borchardt and Benningson, *Münch. med. Wchnschr.*, 1913, LX, 2275; Autenrieth and Montigny, *Ibid.*, 1914, LXI, 1671; Scott, *Am. Jour. Physiol.*, 1914, XXXIV, 271; Bergmark, *Jahrb. f. Kinderhke.*, 1914, LXXX, 373; Lichtwitz, *Berl. klin. Wchnschr.*, 1914, LI, 1018; Broekmayer, *Deutsch. med. Wchnschr.*, 1914, XL, 1562; McLean, *Jour. A. M. A.*, 1914, LXII, 917; Bing and Jakobsen, *Deutsch. Arch. f. klin. Med.*, 1914, CXIII, 571; Menke, *Ibid.*, 1914, CXIV, 209; Müller, *Ztschr. f. physiol. Chem.*, 1914, XCI, 287; Kahler, *Wien. klin. Wchnschr.*, 1914, XXVII, 417; Dresel, *Ztschr. f. exper. Path. u. Therap.*, 1914, XVI, 365; Epstein and Baehr, *Jour. Biol. Chem.*, 1914, XVIII, 21; Wolf and Gutmann, *Ztschr. f. klin. Med.*, 1914, LXXXIX, 394; Hirsch, *Biochem. Ztschr.*, 1915, LXX, 191; *Ztschr. f. physiol. Chem.*, 1915, XCIII, 355; *Ibid.*, 1915, XCIV, 227; Ross and McGuigan, *Jour. Biol. Chem.*, 1915, XXII, 407 and 417; Bass, *Am. Jour. Dis. Child.*, 1915, IX, 63; Wolff, *Deutsch. med. Wchnschr.*, 1915, XLI, 6; Morita, *Arch. f. exper. Path. u. Pharmakol.*, 1915, LXXVIII, 188; Lauritzen, *Therap. d. Gegenw.*, 1915, LVI, 8 and 94; Hopkins, *Am. Jour. Med. Sc.*, 1915, CXLIX, 254; Carlson and Ginsburg, *Am. Jour. Physiol.*, 1915, XXXVI, 28c; Macleod and Pearce, *Ibid.*, 1915, XXXVIII,

not believe the figures are influenced by creatinin, yet Morgulis and Jahr have shown, in their experiments of adding known amounts of creatinin to pure glucose solutions and employing the Benedict method, that quite an appreciable variation does result. By comparison with the new method

415; Jahnson-Blohm, Upsala Lakaref. Forhandl., 1915, XX, 331; Strouse, Stein, and Wisely, Bull. Johns Hopk. Hosp., 1915, XXVI, 211; Geyelin, Arch. Int. Med., 1915, XVI, 975; von Moraczewski, Biochem. Ztschr., 1915, LXXI, 268; Schumm, Ztschr. f. physiol. Chem., 1915, XCVI, 204; Pels, Jour. A. M. A., 1915, LXV, 2077; Niemann, Jahrb. f. Kinderhke., 1916, LXXXIII, 1; Graham, Jour. Physiol., 1916, L, 285; Lee and Scott, Am. Jour. Physiol., 1916, XL, 486; Lombroso, Atti acad. Lincei, 1916, I, 736; Schwartz, Heimann and Mahnen, Jour. Cutan. Dis., 1916, XXXIV, 159; Kleiner, Jour. Expert Med., 1916, XXIII, 507; McCrudden and Sargent, Arch. Int. Med., 1916, XVII, 405; Weston, Jour. Med. Res., 1916, XXXV, 199; Macleod, Jour. Lab. and Clin. Med., 1916, II, 112; Ryser, Deutsch. Arch. f. klin. Med., 1916, CXVIII, 408; Grote, Münch. med. Wchnschr., 1916, LXIII, 1614; Rydgaard, Ugesk. f. Laeger, 1916, LXXXVIII, 2007; Nilsson, Upsala Lakaref. Förhandl., 1916, XXII, 107; Rogers, Boston Med. and Surg. Jour., 1916, CLXXV, 152; Epstein and Aschner, Jour. Biol. Chem., 1916, XXV, 151; Underhill, Ibid., 447, 463 and 471; Epstein, Reiss and Branower, Ibid., XXVI, 25; Murlin and Kramer, Ibid., XXVII, 481, 499 and 517; Hiller and Mosenthal, Ibid., XXVIII, 197; Kuriyama, Ibid., 1917, XXIX, 127; Macleod and Hoover, Am. Jour. Physiol., 1917, XLII, 460; Morriss, Bull. Johns Hopk. Hosp., 1917, XXVIII, 140; Janney, Proc. Soc. Exper. Biol. and Med., 1917, XV, 15; Hamman and Hirschman, Arch. Int. Med., 1917, XX, 761; Epstein and Felsen, Am. Jour. Med. Sc., 1917, CLIII, 58; Cummings and Piness, Arch. Int. Med., 1917, XIX, 777; Denis, Aub. and Minot, Ibid., XX, 964; Mosenthal, Clausen, and Hiller, Ibid., 1918, XXI, 93; McCrudden and Sargent, Ibid., 252; Janney and Isaacson, Ibid., XXII, 160; Ishikawa, Mitt. a.d. Med. Fak. Tokyo, 1918, XIX, 497; Sakaguchi, Hayashi, and Yezima, Ibid., XX, 61; Gettler and St. George, Jour. A. M. A., 1918, LXXI, 2033; Roth, Berl. klin. Wchnschr., 1918, LV, 589; McCrudden and Sargent, Jour. Biol. Chem., 1918, XXXIII, 387; Ross, Ibid., XXXIV, 335; Bandonin Paris Med., 1919, IX, 346; Bailey, Arch. Int. Med., 1919, XXIII, 455; Williams and Humphreys, Ibid., 537, 546 and 559; Edwards, Ind. State. Med. Ass'n. Jour., 1919, XII, 296; Lindblom Hygiea, 1919, LXXXI, 753; Hamman and Hirschman, Bull. Johns Hopk. Hosp., 1919, XXX, 306; Chapin and Myers, Am. Jour. Dis. Child., 1919, XVIII, 555; Sakaguchi, Mitt. a. d. Med. ak. d. Univ. Tokyo, 1918, XX, 345; Büdinger, Deutsch. Arch. f. klin. Med., 1918, CXXVIII, 151; Brinkman and Van Dam, Arch. intern. de physiol., 1919, XV, 105; Grève, I. D., Leiden, 1919; Lindblom, Hygiea, 1919, LXXXI, 753; Alta and Richter-Quittner, Biochem. Ztschr., 1919, C, 148; Bönninger, Ibid., 1920, CIII, 306; Ege, Ibid., 1920, CVII, 229; Ibid., 1920, CXI, 189; Pickering, Quart. Jour. Med., 1920, XIV, 19; Cammidge, Practitioner, 1920, CIV, 114; Goetzky, Ztschr. f. Kinderhke., 1920, XXVII, 195; Rathery and Gruat, Paris Med., 1920, X, 372; Frank and Lotte, Jahrb. f. Kinderhke., 1920, XCI, 313; Silvestri and Aielle, Policlin., 1920, XXVII, 643; Rohdenburg and Pohlman, Am. Jour. Med. Sc., 1920, CLIX, 853; Wilson, Jour. Lab. & Clin. Med., 1920, V, 730; Chabanier and Lebert, Presse Med., 1920, XXVIII, 553; O'Hare, Am. Jour. Med. Sc., 1920, CLX, 366; Maxwell, Med. Jour. Australia, 1920, II, 551; Allen and Wishart, Jour. Biol. Chem., 1920, XLIII, 129; Wishart, Ibid., XLIV, 563; Pemberton and Fester, Arch. Int. Med., 1920, XXV, 243; Delateur, Ibid., 405; Cowie and Parsons, Ibid., XXVI, 333; Strouse, Ibid., 751 and 759; Goto and Kuno, Ibid., 1921, XXVII, 224; Newburgh and Marsh, Ibid., 609; Beeler and Fitz, Ibid., XXVIII, 804; Wooley, Jour. Lab. & Clin. Med., 1921, VI, 227; Maclean and de Wesselow, Quart. Jour. Med., 1921, XIV, 103; Spence, Ibid., 314; Macleod, Physiol. Reviews, 1921, I, 208; Mertz and Reminger, Arch. f. Kinderhke., 1921, LXIX, 81; Graham, Lancet, 1921, I, 1003; Millikin, Am. Jour. Dis. Child., 1921, XXI, 484; Tatum, Jour. Pharmacol. & Exp. Therap., 1921, XVII, 395; van Crefeld, Nederl. Tijdschr. v. Geneesk., 1921, II, 770; McBrayer, Jour. Am. Med. Assoc., 1921, LXXVII, 861; Ambard, Medicine, 1921, II, 936; Cammidge, Forsythe and Howard, Brit. Med. Jour., 1921, II, 586 and 618; Onohara, Brit. Jour. Exp. Path., 1921, II, 104; Partes and Katz-Klein, Ztschr. f. ges. exp. Med., 1921, XXV, 98; Fontès and Thivolle, Bull. soc. chim. biol., 1921, III, 226; Bierry and Rathery, C. R. Acad. des Sc., 1921, CLXXII, 244; Ege, Biochem. Ztschr., 1921, CXIV, 88; Rusznyak and Hetenyi, Ibid., CXXI, 125; Langfeldt, Jour. Biol. Chem., 1921, XLVI, 381, 391 and 403; Fitz and Bock, Ibid., XLVIII, 313; Underhill and Nellaus, Ibid., 557; Wu, Ibid., 1922, LI, 21; Olmsted and Gray, Arch. Int. Med., 1922, XXIX, 384; Opitz, Klin. Wchnschr., 1922, I, 117; Langston, Jour. Lab. & Clin. Med., 1922, VII, 203; Botti, Policlinico, 1922, XXIX, 249; Atkinson and Ets, Jour. Biol. Chem., 1922, LII, 5; Neuwrith and Kleiner, Jour. Lab. & Clin. Med., 1922, VII, 405; Rosenow and Jaguttis, Klin. Wchnschr., 1922, I, 358; Folin and Berglund, Jour. Biol. Chem., 1922, LI, 213; Mann and Magath, Arch. Int. Med., 1922, XXX, 73.

of Folin and Wu, in which picric acid is not employed, it is seen that the figures either by the original Lewis and Benedict method, the modification of Myers and Bailey, or Benedict's last modification are higher.

Method of Folin and Wu.

In the original method of these authors, the blood sugar was determined colorimetrically by the help of the phenol reagent of Folin and Denis. In this determination, the errors due to creatin, creatinin, and uric acid are eliminated, while a new source of error is introduced, namely, that due to the so-called phenols. In the method to be discussed, this latter error has, also, been eliminated, so that we now possess a very exact method for this estimation.¹

Solutions Necessary.

1. *Standard Sugar Solutions.*—Three standard sugar solutions should be on hand: (1) a stock solution of 1 per cent. dextrose or invert sugar preserved with xylol or toluol; (2) a solution containing 1 mg. of sugar per 10 c.c. (5 c.c. of the stock solution diluted to 500 c.c.); (3) a solution containing 2 mg. of sugar per 10 c.c. (5 c.c. of the stock solution diluted to 250 c.c.).

2. *Alkaline Copper Solution.*—Dissolve 40 grams of anhydrous sodium carbonate in about 400 c.c. of water and transfer to a liter flask. Add 7.5 grams of tartaric acid and when the latter has dissolved, add 4.5 grams of crystallized copper sulphate. Mix and make up to a volume of 1 liter. If the chemicals used are not pure, a sediment of cuprous oxid may form in the course of 1 or 2 weeks. If this should happen, remove the clear supernatant reagent with a siphon or filter through a good quality filter paper.

3. *Molybdate-Phosphate Solution.*—Transfer to a liter beaker 35 grams of molybdic acid and 5 grams of sodium tungstate. Add 200 c.c. of 10 per cent. sodium hydrate and 200 c.c. of water. Boil vigorously for 20 to 40 minutes so as to remove nearly the whole of the ammonia present in the molybdic acid. Cool, dilute to about 350 c.c. and add 125 c.c. of concentrated (85 per cent.) phosphoric acid. Dilute to 500 c.c.

Technic.

Transfer 2 c.c. of the tungstic acid blood filtrate (corresponding to 0.2 c.c. of blood) discussed under non-protein nitrogen to a special blood sugar test-tube,² and to two other similar test-tubes (graduated at 25 c.c.) add 2 c.c. of standard sugar solution containing respectively 0.2 and 0.4 mg. of dextrose. To each tube add 2 c.c. of the alkaline copper solution. The surface of the mixtures must now have reached the constricted part of the tubes. If the bulb of the tube is too large for the volume (4 c.c.) a little, but

¹ As the sugar content of blood is prone to decrease even when preserved at low temperatures, Denis and Aldrich (Jour. Biol. Chem., 1920, XLIV, 203) advise the addition of 1 drop of formalin to 5 c.c. of oxalated blood as a preservative, control experiments showing little or no variation in sugar content after 96 hours by this treatment.

² The special blood sugar test-tubes, as devised by Folin and Wu, may be obtained from Emil Greiner Company, 55 Fulton St., New York, or from the Arthur H. Thomas Company, West Washington Square, Philadelphia. These tubes consist of a Pyrex tube graduated at 25 c.c. and constricted toward the bottom in such a manner as to form a bulb which will contain 4 c.c., the fluid rising just to the constricted portion of the tube when 4 c.c. are present. The constriction is 8 mm. wide and 4 cm. in length.

not more than 0.5 c.c. of a diluted (1 : 1) alkaline copper solution may be added. If this does not suffice to bring the contents to the narrow part, the tube should be discarded. Test-tubes having so small a capacity that 4 c.c. fills them above the neck should also be discarded. Transfer the tubes to a boiling water bath and heat for 6 minutes. Then transfer them to a cold water bath and let them cool, without shaking, for 2 or 3 minutes. Add to each test-tube 2 c.c. of the molybdate-phosphate solution. The cuprous oxid dissolves rather slowly if the amount is large, but the whole, up to the amount given by 0.8 mg. of dextrose, dissolves usually within 2 minutes. When the cuprous oxid is dissolved, dilute the resulting blue solutions to the 25 c.c. mark, insert a rubber stopper, and mix. It is essential that adequate attention be given to this mixing because the greater part of the blue color is formed in the bulb of the tube. The color comparisons are made in the usual manner. The depth of the standard in mm. multiplied by 100 and divided by the reading of the unknown gives the sugar in mg. per 100 c.c. of blood, when the lower standard is employed; while the depth of the standard must be multiplied by 200 when the solution containing 0.4 mg. is employed.

The two standards given, representing 0.2 and 0.4 mg. of glucose, are adequate for practically all cases, as they cover the range from about 70 to nearly 400 mg. of glucose per 100 c.c. of blood. In a series of 40 cases, reported by Folin and Wu, the range of blood sugar was from 70 to 170 mg. per 100 c.c. of blood. It is interesting to note that the parallel figures for creatinin and sugar in the same cases ran as follows, notations being taken from different parts of the table: Total creatinin, 6.1 mg., sugar 119 mg.; total creatinin, 19.4 mg., sugar 99 mg.; total creatinin, 20.5 mg., sugar, 170 mg.; total creatinin, 27.2 mg., sugar, 157 mg. per 100 c.c. of blood. This newer method of Folin and Wu is to be regarded as superior to the earlier method as it eliminates the possibility of reoxidation of the cuprous compounds as well as the error due to the so-called phenols in the blood filtrate.¹

¹ Folin and Wu, Jour. Biol. Chem., 1920, XLI, 367. For the original method of Folin and Wu see Jour. Biol. Chem., 1919, XXXVIII, 106. For other methods and points in connection with these methods see Kowarski, Deutsch. med. Wchnschr., 1913, XXXIX, 1635; Bang, Biochem. Ztschr., 1913, XLIX, 10; Münch. med. Wchnschr., 1913, LX, 2277; Dorner, Ztschr. f. klin. Med., 1914, LXXIX, 287; Kraus, Lancet, 1914, I, 1249; Kaminura, Sei-I-Kwai, 1914, XXXIII, 38; Gardner and Maclean, Biochem. Jour., 1914, VIII, 391; Maasa and Tachau, Ztschr. f. klin. Med., 1914, LXXI, 1; Fitz, Arch. Int. Med., 1914, XIV, 133; Epstein, Jour. A. M. A., 1914, LXIII, 1667; Schaffer, Jour. Biol. Chem., 1914, XIX, 285; Lewis and Benedict, Ibid., 1915, XX, 61; Pearce, Ibid., XXII, 525; Kahn, Jour. A. M. A., 1915, LXIV, 241; Ryser, Deutsch. Arch. f. klin. Med., 1915, CXVIII, 316; Myers and Bailey, Jour. Biol. Chem., 1916, XXIV, 147; Morris, Jour. Lab. and Clin. Med., 1916, I, 252; Macleod, Ibid., 445; McDaniel, Ibid., 804; McGuigan, Ibid., 1917, II, 514; Ege, Ugesk. f. Laeger, 1917, LXXIX, 120; McGuigan and Ross, Jour. Biol. Chem., 1917, XXXI, 533; Benedict, Ibid., 1918, XXXIV, 203; Addis and Shevsky, Ibid., XXXV, 43 and 53; Egerer, Ibid., 565; Rohde and Sweeney, Ibid., XXXVI, 475; Benedict, Ibid., 1919, XXXVII, 503; Morgulis and Jahr, Ibid., XXXIX, 119; Maclean, Biochem. Jour., 1919, XIII, 135; de Wesselow, Ibid., 148; Yamakami, Am. Jour. Physiol., 1919, L, 177. Hagedorn and Jensen, Ugesk. f. Laeger, 1918, LXXX, 1217; Kowarsky, Deutsch. med. Wchnschr., 1919, XLV, 188; Vigewani, Boll. chim. farm., 1919, LVIII, 436; Stepp, Ztschr. f. physiol. Chem., 1919, CVII, 120; Biochem. Ztschr., 1920, CVII 60; Cammidge, Practitioner, 1920, CIV, 114; Neau, Theses, Montpellier, 1920; Bahlmann, Nederl. Tijdschr. v. Geneesk., 1920, I, 1820; Host and Hatlehol, Norsk. Mag. f. Laegevidensk., 1920, LXXXI, 877; Wallis and Gallagher, Lancet, 1920, II, 784; Borde, Bull. soc. pharm. de Bordeaux.

Benedict's Method.

This method¹ is a modification of the one of Lewis and Benedict, which had the drawback that it was necessary to boil to dryness in a test-tube to complete the reaction between the sugar and picric acid.

Two c.c. of blood are drawn into an Ostwald pipet, containing a little powdered potassium oxalate and discharged into a 25 c.c. graduated flask, or into a large test-tube graduated at 12.5 and at 25 c.c. The pipet is twice rinsed out with distilled water, these washings being added to the blood. After a minute or two the blood is practically completely laked. A solution of sodium picrate and picric acid² is added to the 25 c.c. mark (using a few drops of alcohol to dispel foam if necessary) and the mixture thoroughly shaken. After a minute or two (or longer) the mixture is poured upon a dry filter, and the clear filtrate collected in a dry beaker. Exactly 8 c.c. of the filtrate are measured into a large test-tube bearing graduations at the 12.5 c.c. and 25 c.c. mark, and 1 c.c. of 20 per cent. (anhydrous) sodium carbonate solution is added. The tube is plugged with cotton and immersed in boiling water for 10 minutes (longer heating up to 12 hour makes no change in color). It is then removed and the contents are cooled under running water and diluted to 12.5 c.c. or to 25 c.c. depending on the depth of color. At any time within $\frac{1}{2}$ hour the colored solution is compared in a colorimeter with a suitable standard solution, the standard being set at a height of 15 mm. Occasionally the final filtrates in this method develop a little turbidity during heating. Unless this be fairly marked it is of no consequence. If desired filter the final colored solution through a small folded filter into the colorimeter cup.

Standard Solution.—The standard solution may be simultaneously prepared from pure glucose by treating 0.64 mg. of glucose in 4 c.c. of water with 4 c.c. of the picrate-picric acid solution and 1 c.c. of the 20 per cent. sodium carbonate solution, and heating for 10 minutes in boiling water and then diluting to 12.5 c.c.

A permanent standard solution may be prepared from a stock solution

1920, LVIII, 214; Guillaumin, Jour. pharm. chim., 1920, XXII, 327 and 378; Höst and Hatlehol, Jour. Biol. Chem., 1920, XLII, 347; Kleiner, Jour. Am. Med. Assoc., 1921, LXXVI, 172; Napveux, Bull. Med., 1921, XXXV, 616; Ionescu and Vărglobei, Bull. soc. chim. Romania, 1921, II, 102; Stepp, Arch. exp. Path. u. Pharm., 1921, XC, 105; Ponder and How'e, Biochem. Jour., 1921, XV, 171; Cooper and Walker, Ibid., 415; Guy, Ibid., 575; Scriver, Nederl. Tijdschr. v. Geneesk., 1921, II, 2534; Stammers, Med. Jour. Australia, 1922, XVII, 139; Baumann and Isaacson, Jour. Lab. & Clin. Med., 1922, VII, 357; John, Jour. Am. Med. Assoc., 1922, LXXVIII, 103; Thalhimer and Updegraff, Ibid., 1383; Pollock and McEllory, Am. Jour. Med. Sc., 1922, CLXIII, 571.

¹ Jour. Biol. Chem., 1918, XXXIV, 203; Lewis and Benedict, Ibid., 1915, XX, 61.

² Place 36 grams of dry powdered picric acid (or 40 of the usual moist article on the market) in a liter flask or stoppered cylinder, add 500 c.c. of 1 per cent. sodium hydroxid solution and 400 c.c. of hot water. Shake occasionally until dissolved. Cool and dilute to 1 liter. It may happen, occasionally, that this picrate solution will not precipitate properly the proteins of the blood. This is due, as Benedict (Jour. Biol. Chem., 1910, XXXVII, 503) has shown, either to impurity in the picric acid or to addition of a little too much alkali during preparation of the solution. For proper precipitation of the blood, this picrate solution must have an acidity as high as 0.05 or 0.04 NN, as determined by titration of a portion of the solution with alkali, using phenolphthalein as indicator. The correction may be made by addition of a quantity of glacial acetic acid calculated to bring the acidity between the above points. Any excess of acid over that necessary is to be avoided.

containing 100 mg. of picramic acid and 200 mg. of sodium carbonate per liter. 12.6 c.c. of this solution are treated with 1 c.c. of the 20 per cent. carbonate solution and 15 c.c. of the picrate picric acid solution, and diluted to 300 c.c. with distilled water.

Calculation.—
$$\frac{\text{Reading of Standard}}{\text{Reading of unknown}} \div 10 = \text{per cent. of sugar in the original blood.}$$
 Where the final dilution is made to 25 c.c. instead of 12.5, the final figure is multiplied by 2.

Glycogen.

This polysaccharid undoubtedly appears in the blood singly or in combination with albuminous bodies. Salomon, Frerichs, Lepine, Ehrlich, and Gabritschewsky have reported it, while Caminer was unable to find it. Hup-pert obtained it in quantities ranging from 0.114 to 1.56 grams per 100 parts of blood. Much depends on the method used to isolate and determine this substance, as it is easily lost by careless manipulation. Certain properties of the granules found in the blood, both extra- and intracellularly, have led many to believe that glycogen is present as a characteristic in many conditions. There is much reason, however, to assume that these granules, which stain brownish with iodine, are not glycogen, but rather albuminous bodies of indefinite composition. For a discussion of this subject see the treatment of iodophilia.

(F) Fats and Fatty Acids.

The presence of free fats (palmitin, stearin, and olein) in the blood has been frequently observed both in health and disease. The relative quantities of these fats vary in different animals and are subject to wide variation in the same animal under influence of diet. The total lipid content of the blood under normal conditions would appear to be about 0.6 per cent., being higher under influence of digestion.

The presence of an excess of free fat in the blood is referred to as lipemia. The physiologic variations are more notable than are the pathological, being observed after ingestion of a meal rich in fats, in breast-fed children, in pregnant women, and in the obese.¹ Pathologically lipemia more or less permanent may be observed in various conditions, as in acute and chronic alcoholism, diabetes, arteriosclerosis, chronic nephritis, phthisis, carbon monoxide and phosphorous poisoning, gout, typhoid fever, fat embolism following injuries of the long bones, pneumonia, leukemia, acute infections, cachexia from inanition or malignant disease, hepatic diseases, and malaria. It has, therefore, little differential diagnostic value.

¹ Hermann and Neumann, *Biochem. Ztschr.*, 1911, XLIII, 47; Bosco, *Policlinico*, 1914, XXI, 314 and 357; Sakai, *Biochem. Ztschr.*, 1914, LXII, 387; Klein and Dinkin, *Ztschr. f. physiol. Chem.*, 1914, XCII, 302; Greenwald, *Jour. Biol. Chem.*, 1913, XIV, 369; *Ibid.*, 1915, XXI, 20; *Am. Jour. Med. Sc.*, 1914, CXLVII, 225; Bloor, *Jour. Biol. Chem.*, 1914, XVII, 377; *Ibid.*, 1914, XIX, 1; *Ibid.*, 1915, XXII, 133; Imrie, *Ibid.*, 1915, XX, 87. It is interesting to note that the lowest normal value for cholesterol obtained by Denis (0.167 per cent.) was found in a woman weighing 200 pounds. See eigl, *Biochem. Ztschr.*, 1910, XC, 173; Horiuchi, *Jour. Biol. Chem.*, 1920, XLIV, 345; Blatherwick, *Ibid.*, 1921, XLIX, 193; Bloor, *Ibid.*, 201; Lemoland, *Bull. soc. chim. biol.*, 1921, III, 134; Bloor, Palkan and Allen, *Jour. Biol. Chem.*, 1922, LII, 191; Henning, *Ibid.*, LIII, 167.

The extent of the lipemia may vary from the presence of isolated fat droplets to the overloading of the blood to such a degree that it becomes salmon-colored, turbid, and milky. This fat may be either the normal fat which has been transported from different parts of the body or may be that abnormal to the body arising from excess of fat in the diet. It is possible that a marked decrease in the lipase of the blood may be a factor in the production of lipemia. The fat is soluble in ether and stains black with osmic acid and red with Sudan-III. Regarding the quantitative estimations of fat, I must refer to other sections, the Gephart-Csonka method¹ having been applied to blood fats with good results.

Concerning the presence of fatty acids in the blood little is known. Traces of volatile acids are sometimes present, but probably not as normal constituents. Gaglio, Spiro, Irisawa, and Berlinerblau report sarcolactic acid as a normal finding, while Zweifel finds an excess of this acid in the blood and in the urine in cases of toxemia of pregnancy. An excess of fatty acids in the blood is known as lipacidemia. Von Jaksch found fatty acids in the blood in cases of diabetic coma, leukemia, acute yellow atrophy of the liver, while Hougounenq reports the presence of β -oxybutyric acid in the cadaveric blood of a diabetic. It is rational to assume that in all those conditions associated with acidosis, fatty acids are present in the blood, as these may be detected in large amount in the urine in these states. Bloor and MacPherson² show that the total fatty acids of whole blood averages 0.36 per cent., while in the plasma the value is 0.38 per cent. The figures of Csonka show a normal fatty acid average of 0.297 per cent., the unsaturated type forming 48 per cent. of the total. This latter worker shows, also, that the blood lipid values in anemia are normal so long as the percentage of corpuscles remains above $\frac{1}{2}$ the normal figures; below such percentage, the plasma shows a high total fat with low cholesterin and lecithin values.

Cholesterol.

In recent times the variations in the amount of cholesterin in the blood have assumed considerable importance. It is, beyond doubt, true that cholesterin is present in increased amounts both associated with lipemia and not so associated. The cholesterinemia of the alimentary type appears to be dependent on the intake of cholesterin-containing food. While the figures for the amount of cholesterin in the blood vary to a slight extent, a fairly accurate normal standard may be taken as 150 mg. per 100 c.c. of blood, although the average figures of Bloor are 0.21 per cent., while those of Denis range from 0.167 to 0.255 per cent. and of Gorham and Myers from 0.13 to 0.19 per cent. The results of Bloor and Knudson indicate that the cholesterol esters form 33.5 per cent. of the total cholesterin in whole blood and 58 per cent. of that in the plasma.

A hyper-cholesterinemia obtains in pregnancy, just as does a lipemia. The cholesterin gradually rises during gestation, reaches its maximum in the

¹ Jour. Biol. Chem., 1914, XIX, 521; see also, Csonka, *Ibid.*, 1918, XXXIII, 401.

² Jour. Biol. Chem., 1917, XXXI, 79; Csonka, *Ibid.*, 1918, XXXIII, 401; Laquer, *Ztschr. f. Biol.*, 1919, LXX, 99; Harrop, *Proc. Soc. Exp. Biol. and Med.*, 1920, XVII, 2126.

last months of pregnancy and drops to normal in 8 to 10 days after delivery, irrespective of lactation. In the eclampsias of pregnancy very high values are noted. In chronic nephritis an increase is observed, the degree running parallel to the severity of the case. This depends on the averages taken as normal values, as Denis states that cholesterol remains at normal levels although as high values as 0.29 per cent. are reported by her while Gorham and Myers report a hypercholesterolemia, with values usually running about 0.2 per cent. In arteriosclerosis an increase of greater or less extent is practically always observed. In diabetes mellitus and in tuberculosis one obtains relatively high values as a rule. Very high figures (up to 900 mg. per 100 c.c.) are observed in cholelithiasis, a point of some value in the study of pathogenesis of gall-stones.¹

An interesting fact is observed in the study of the blood of febrile cases, especially of typhoid fever, pneumonia, scarlet fever, etc. In such cases it is the rule to find a diminution of cholesterin, the higher the fever the less the cholesterin content of the blood. As the temperature diminishes, the cholesterin gradually rises, returning to about the normal point as a rule. If the fever be long continued, the cholesterin rise in the period of defervescence goes quite appreciably above the normal values.

¹ Lifschütz, Ztschr. f. physiol. Chem., 1907, L, 437; Ibid., 1907, LIII, 140; Ibid., 1908, LVIII, 175; Ibid., 1909, LXIII, 223; Biochem. Ztschr., 1913, LII, 206; Ztschr. f. physiol. Chem., 1914, XCI, 309; Ibid., 1914, XCII, 383; Ibid., 1914, XCIII, 209; Windaus, Ibid., 1910, LXII, 174; Ibid., 1910, LXV, 110; Hanes, Bull. Johns Hopkins Hosp., 1912, XXIII, 77; Weston, Jour. Med. Research, 1912, XXVI, 47; Weston and Kent, Ibid., 531; Henes, Deutsch. Arch. f. klin. Med., 1913, CXI, 122; Jour. Am. Med. Assn., 1914, LXIII, 146; Thaysen, Biochem. Ztschr., 1914, LXII, 89 and 115; von Czychlarz and Fuchs, Ibid., 131; Antonelli, Policlinico, 1914, XXI, 341; Farini, Gazz. d. osp., 1914, XXXV, 993; Fischl, Wien. klin. Wchnschr., 1914, XXVII, 982; Anitschkow, Med. Klin., 1914, X, 465; Pribram, Zentralbl. f. inn. Med., 1914, XXXVI, 325; Quinan, Calif. State Jour. Med., 1914, XII, 118; Weiss, New York Med. Jour., 1914, C, 180; Chauffard and Grigaut, Presse méd., 1914, XXI, 929; Milkwitsch, Russk. Vrach, 1914, XXII, 1911; Cannata, Pediatria, 1915, XXIII, 161; Hußmann, Zentralbl. f. Gynäk., 1915, XXXIX, 33; Henes, New York State Jour. Med., 1915, XV, 310; Mueller, Jour. Biol. Chem., 1915, XXII, 1; Bloor, Ibid., XXIII, 317; Cruickshank and Tisdall, Jour. Mental Sci., 1916, LXII, 168; Macleod, Jour. Lab. and Clin. Med., 1916, I, 529; Luden, Ibid., 662; Small, Ibid., 809; Hymanson and Kahn, Am. Jour. Obs., 1916, LXXIII, 1041; de Langen, Presse Méd., 1916, XXIV, 332; Dewey, Trans. Chic. Path. Soc., 1916, X, 52; Henes, Surg. Wyn. and Obs., 1916, XXIII, 91; Goñalons, Sem. Med., 1916, XXIII, 408 and 639; Wahl and Richardson, Arch. Int. Med., 1916, XVII, 238; Dewey, Ibid., 1916, XVII, 757; Dubin and Pearce, Ibid., XVIII, 426; Bloor, Jour. Biol. Chem., 1916, XXIV, 227; XXV, 577; XXVI, 417; Luden, Ibid., XXVII, 273; Jour. Lab. and Clin. Med., 1917, III, 93 and 141; Denis, Jour. Biol. Chem., 1917, XXIX, 93; Bloor and MacPherson, Ibid., XXXI, 79; Bloor, Ibid., 575; Knudson, Ibid., XXXII, 337; Gorham and Myers, Arch. Int. Med., 1917, XX, 599; Csonka, Jour. Biol. Chem., 1918, XXXIII, 401; Luden, Jour. Lab. and Clin. Med., 1918, IV, 849; Giesens, Deutsch. Arch. f. klin. Med., 1918, CXXVII, 439; Stepp, Münch. med. Wchnschr., 1918, LXV, 781; Burge and Reinhart, Ztschr. f. exp. Med., 1918, VII, 119; Bang, Biochem. Ztschr., 1918, XC, 383; Ibid., XCI, 104, 111, 122 and 224; Feigl, Ibid., XCII, 282; Bang, Ibid., 1919, XCIV, 359; Rothschild and Felsen, Arch. Int. Med., 1919, XXIV, 520; Luden, Jour. Lab. and Clin. Med., 1919, IV, 719; Pacini, Med. Record, 1919, XCIV, 441; Yakakoshi, Japan Med. World, 1919, 304; Cordier, Boulub, and Colrat, Jour. d'Urol., 1920, IX, 81; Myers, Jour. Lab. & Clin. Med., 1920, V, 776; Richter-Quittner, Wiener Arch. f. inn. Med., 1920, I, 425; Honos, Arch. Int. Med., 1920, XXV, 411; Bailey and MacKay, Ibid., 628; Arning and Lippmann, Ztschr. f. klin. Med., 1920, LXXXIX, 107; Feigl, Ztschr. f. exp. Med., 1920, XI, 178; Boom, I.D., Amsterdam, 1920; Beumer, Monatsschr. f. Kinderhke., 1920, XVIII, 443; Arch. f. Kinderhke., 1920, LXVIII, 105; Chauffard, Laroche and Grigaut, Ann. de Méd., 1920, VIII, 69 and 149; Kipp, Jour. Biol. Chem., 1920, XLIII, 413; XLIV, 215; Chauffard and Troisier, Ann. de Méd., 1921, IX, 149; Malerba, Rif. Med., 1921, XXXVII, 602; Alessandri, Ibid., 1905; Hahn and Wolff, Ztschr. f. klin. Med., 1921, XCII, 393; Gaudissart, Am. Jour. Ophthalmol., 1922, V, 118.

Determination of Total Cholesterol.

Method of Bloor.¹

Preparation of Sample.

Three c.c. of whole blood, plasma, or serum are run slowly (a slow stream of drops) from a pipet into about 75 c.c. of a mixture of redistilled alcohol and ether (3 parts alcohol, 1 part ether) in a 100 c.c. graduated flask. The contents of the flask should be kept in motion during the process so that there is no clumping of the precipitated material. The contents of the flask are raised to boiling by immersion in a water bath (with constant stirring to avoid superheating), cooled to room temperature, filled to the mark with alcohol-ether, mixed, and filtered. The filtered liquid, if placed in a tightly stoppered bottle in the dark, will keep unchanged for a considerable time. By running the blood slowly into the large quantity of alcohol-ether, as directed above, the protein material is precipitated in finely divided form and, under these conditions, the short heating combined with the great excess of solvent is adequate for complete extraction of serum or plasma. With whole blood, the results are not quite so complete.

Technic.

Ten c.c. of the above alcohol-ether extract are measured into a small flat-bottomed beaker and evaporated *just* to dryness on a water bath. Any heating, after drying is reached, produces a brownish color which passes into the chloroform used later and renders the subsequent determination difficult or impossible. This point must be remembered. The cholesterol is extracted from the dry residue by boiling out three or four times with successive small portions of chloroform and decanting into a 10 c.c. glass-stoppered, graduated cylinder, which has previously been calibrated. (In this extraction an excess (4 or 5 c.c.) should be added each time and the mixture allowed to boil down to somewhat less than $\frac{1}{2}$ its volume before decanting). The combined extracts, after cooling are then made up to 5 c.c. The solution should be colorless, but not necessarily clear, since the slight turbidity clears up on adding the reagents.

Five c.c. of a standard cholesterol solution in chloroform (containing 0.5 mg. of cholesterol) are measured into a similar 10 c.c. cylinder. (It is convenient to make the cholesterol standard in two strengths: (a) the stock solution containing 0.2 gram of pure cholesterol in 200 c.c. chloroform; and (b) the standard solution for use, made by diluting 10 c.c. of the above to 100 c.c. with chloroform. Five c.c. of this latter solution will contain 0.5 mg.)

To each of the solutions in the 10 c.c. cylinders are added 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulphuric acid (in order to produce

¹ Jour. Biol. Chem., 1916, XXIV, 227. See, also, Csonka, *Ibid.*, 431; Mueller, *Ibid.*, XXV, 549; Hoover and Blankenhorn, *Arch. Int. Med.*, 1916, XVIII, 289; Weston, *Jour. Biol. Chem.*, 1917, XXVIII, 383; Bloor, *Ibid.*, XXIX, 437; Luden, *Ibid.*, 463; Csonka, *Ibid.*, 1918, XXXIV, 577; Bernhard, *Ibid.*, XXXV, 15; Myers and Wardell, *Ibid.*, XXXVI, 147; Windaus, *Nachr. kgl. Ges. Göttingen*, 1919, 237; Fox, *Biochem. Ztschr.*, 1920, CIV, 82; Rosenthal and Holzer, *Ibid.*, CVIII, 220; Csonka, *Jour. Biol. Chem.*, 1920, XLI, 243; Knudson, *Ibid.*, 1921, XLV, 255; Gardner and Williams, *Biochem. Jour.*, 1921, XV, 363; Gardner and Fox, *Ibid.*, 376.

the Liebermann-Burchard color reaction), the solutions are mixed by inverting several times, then set away in the dark for 15 minutes, after which they are transferred to the cups of the colorimeter and compared as usual, setting the standard at 15 mm. The cement of the colorimeter cups is soluble in chloroform, so that a coating of plaster of Paris must be used to avoid solution.

On account of the adverse criticism of this method by Weston and the determinations of Bloor, himself, on the use of standard solutions of cholesterol at different temperatures in making these color comparisons, Bloor has advised that this comparison be made at approximately 22°C., owing to the different behavior of the blood cholesterol as compared with the standard. It is recommended, therefore, that, instead of using the cholesterol solution as a standard, a solution of naphthol green B be employed as employed by Gorham and Myers. The standard solution of this dye is made by diluting 2 c.c. of a 0.1 per cent. aqueous solution to 17 c.c. with distilled water, obtaining a 0.0118 per cent. solution. This solution has a colorimetric strength, which is practically identical with that obtained from a 0.08 per cent. chloroform solution of cholesterol and is, therefore, somewhat stronger than the standard solution of Bloor.

This method of Bloor gives somewhat higher results than those by his first or saponification method. As Luden has shown, this may be due to certain bile derivatives other than cholesterol. She suggests that parallel determinations by the two methods be employed supplementing these by employing the dialyzation method of eliminating the bile salts and pigments as advocated by Hoover and Blankenhorn. The method of Myers and Wardell gives lower values than that of Bloor.

Determination of the Cholesterol Esters.

Method of Bloor and Kundson.¹

Twenty c.c. of the alcohol-ether extract, obtained as in the method above (or sufficient to contain about 0.5 mg. of combined cholesterol) are measured into a small flat-bottomed Erlenmeyer flask (50 c.c.) and 1 c.c. of a 1 per cent. alcoholic solution of digitonin is added. The whole solution is then evaporated just to dryness on the water bath. The digitonin combines with the free cholesterol forming digitonin cholesterolide while the cholesterol present as ester is not affected. The dried residue in the flask is then extracted by boiling out with successive small portions of petroleum ether (boiling point below 60°C.), filtering the extract through a plug of fat-free cotton in the stem of a small funnel. In order to get a complete extraction with a small amount of solvent, 15 c.c. of the petroleum ether are first added, the flask is covered with a small watch glass (to prevent too rapid evaporation) and the whole boiled gently until about half the liquid is gone. The succeeding extractions are made in a similar manner with 7 to 8 c.c. of petroleum ether, which dissolves the cholesterol esters but not the digitonin precipitate. The combined extracts are then evaporated just to dryness, and the esters taken up with chloroform as in the above method of Bloor. These

¹ Jour. Biol. Chem., 1916, XXVIII, 107; Ibid., 1917, XXIX, 7.

chloroform extracts, measuring slightly less than 5 c.c., are collected in a 10 c.c. glass-stoppered cylinder, cooled, and made up to 5 c.c. The remainder of the determination is as given above for the determination of total cholesterol.

(G) Acetone.

The occurrence in the blood of demonstrable amounts of acetone is known as acetonemia. Deichmüller and von Jaksch have found a substance giving the reaction of acetone in various conditions, especially in fevers, which finding has been confirmed by Reale. Whether acetone is a product of normal intermediary metabolism and, as such, is found in many physiologic and pathologic states, must be found in the section on Urine.¹

(H) Biliary Constituents.

The conditions in which the biliary constituents, especially the pigments and acids, are found in the blood is termed cholemia.² It is usually stated that these elements are not found in normal blood, but Croftan has shown that the bile acids are observed in the blood of healthy subjects. This is not unexpected as they are completely absorbed from the intestines and are re-excreted in the bile (Weintraud).³

Pathologically, both the acids and the pigments are found in the blood in any condition associated with their appearance in the urine. Oftentimes they may be found in the blood when no reaction for them is obtainable in the urine. While the bile acids exert marked toxic effects, such as hemolysis, the biliary pigments show, as Bouchard, de Bruin, Lugli, and Colosanti have demonstrated, certain harmful influences. Flint ascribes the toxic effects observed in cholemia to cholesterin, but this idea needs confirmation. The most usual condition showing cholemia is jaundice. Whether the blood changes observed in the various types of jaundice are due, primarily, to the cholemia or to a primary hemolysis or to a combination of these effects as a result of intoxication is an unsettled question. Hijmans van den Bergh has studied the question of jaundice from the standpoint of the presence of biliary substances in the blood, while Brulé attacks the problem from the urinary side. The former has introduced a modification of the diazo reaction for this work as follows: The blood serum is treated with twice its volume of alcohol and the albumin centrifuged out. The supernatant fluid is drawn off with a pipet and the reagent is added in the proportion of 25 per cent. by volume. (This reagent is made fresh for the tests and consists of 25 c.c. of a 1:1000 aqueous solution of sulphanilic acid, 15 c.c. concentrated HCL, and

¹ See Marriott, *Jour. Biol. Chem.*, 1914, XVIII, 507; *Jour. Am. Med. Assn.*, 1914, LXIII, 397; Scheel, *Ugesk. f. Laeger*, 1910, LXXVIII, 905; Moore, *Am. Jour. Dis. Child.*, 1910, XII, 244; Van Slyke and Fitz, *Jour. Biol. Chem.*, 1917, XXXII, 405; *Ibid.*, 1919, XXXIX, 23; Ljungdahl, *Biochem. Ztschr.*, 1910, XCVI, 345; Widmark, *Biochem. Jour.*, 1910, XIII, 430; Marfan, *Arch. de Méd. des Enfants*, 1921, XXIV, 5; Hubbard, *Jour. Biol. Chem.*, 1921, XLIX, 375; Hubbard and Wright, *Ibid.*, 385; Remond, *Bull. de l'Acad. de Méd.*, 1922, LXXXVII, 321.

² See Lehdorff, *Prager med. Wchnschr.*, 1912, XXXVII, 495.

³ Hijmans v. d. Bergh and Snapper (*Deutsch. Arch. f. klin. Med.*, 1913, CX, 540) show that the serum always contains traces of bilirubin along with lutein. See, also, Whipple and Hooper, *Jour. Exper. Med.*, 1913, XVII, 593 and 612.

0.75 c.c. of a 0.5 per cent. solution of sodium nitrite.) In the presence of bilirubin the fluid turns red with a violet tinge. The originator claims that this test will detect bilirubin in a concentration as low as 1:1,500,000. He finds, by applying this test, that there are two sorts of bilirubin in the blood serum, or at least that it reacts in different ways. The bilirubin of mechanical icterus reacts with the diazo reagent in the uncoagulated serum (direct reaction), is largely absorbed by the coagulum of proteins formed by alcohol, oxidizes more readily and is more readily excreted than bilirubin of the other type; which facts account for the finding that in hemolytic jaundice bilirubin is not excreted in the urine but only urobilin. The second type of bilirubin gives the diazo reaction only when the serum has been coagulated by alcohol (indirect reaction). In normal serum a small amount of this type of bilirubin is constantly present. It is considerably increased in pernicious anemia, while there is usually a low level of bilirubin in carcinoma. Difficulties may arise in diagnosis when metastases have occurred in the liver, which may bring about a mechanical jaundice, but, under these conditions, a direct reaction may serve as an early sign of such metastasis.¹

(I) Inorganic Constituents.

The inorganic composition of the blood shows quite a marked variation both under physiologic and pathologic influences. This variation applies both to the cellular and intracellular constituents of the blood. Changes in the molecular concentration as well as changes in the concentration of specific inorganic combinations are observed in the blood of the two sexes, as may be seen by consulting the table on page 467.

Regarding the special significance of the different inorganic constituents little is known, but chemical analyses of the blood ash in health and disease have shown that the pathological variations are more important as regards the chlorids, phosphates, and the calcium and iron compounds.²

Chlorids.

Physiologically, a certain (about six parts per mille) concentration of sodium chlorid is necessary to hold the proteins in solution, as well as to maintain the proper osmotic tension of the serum. The larger the proportion of plasma, the greater the percentage of chlorids in the blood. This is true only within certain limits as the NaCl-content remains practically constant,

¹ See Pel, *Deutsch. Arch. f. klin. Med.*, 1912, CVI, 239; also, Brugsch and Retzlaff, *Ztschr. f. exper. Path. u. Therap.*, 1912, XI, 508; Barratt and Yorke, *Ann. Trop. Med. and Parasitol.*, 1914, VIII, 509; Fischer, *Ztschr. f. physiol. Chem.*, 1916, XCV, 78; Blankenhorn, *Arch. Int. Med.*, 1917, XIX, 344; Bauer and Spiegel, *Deutsch. Arch. f. klin. Med.*, 1919, CXXIX, 17; Hijmans van der Bergh, "Der Gallenfarbstoff im Blute, 1918; Brule, *Recherches recentes sur les ictères*, 1919; Gilbert, Chabrol and Bonard, *C. R. soc. de biol.*, 1920, LXXXIII, 1602; Feigl and Inerner, *Ztschr. f. exp. Med.*, 1919, IX, 153; Botzian, *Mitt. a. d. Grenzgeb., d. Med. u. Chir.*, 1920, XXXII, 540; Haselhorst *Münch. med. Wochenschr.*, 1921, LXVIII, 175; Lepohne, *Deutsch. Arch. f. klin. Med.*, 1921, CXXXV, 79; Hijmans van der Bergh, *Presse Med.*, 1921, XXIX, 441; Hellmuth, *Monatsschr. f. Geb. u. Gynäk.*, 1921, LIV, 341; Thannhauser and Anderson, *Deutsch. Arch. f. klin. Med.*, 1921, CXXXVII, 179; Jones, *Arch. Int. Med.*, 1922, XXIX, 643; McNee, *Brit. Med. Jour.*, 1922, I, 716; Vogl and Zins, *Med. Klin.*, 1922, XVIII, 667.

² See Weissbein and Aufrecht, *Internat. Beitr. z. Path. u. Therap. d. Ernährungsstör.*, 1912, IV, 22.

no matter how large an amount is ingested. This constant value is regulated by the increased or diminished renal excretion.¹

In anemias the chlorids of the blood are usually high, yet, according to Limbeck, cases are occasionally found in which normal amounts of sodium chlorid obtain. In pneumonia, in which a diminished urinary excretion of chlorids is observed, the blood does not show an excess of chlorids but may even show a decrease owing to the effects of the exudative process. Diminished ingestion of food, vomiting, diarrhea, and general exudative processes may be associated with a decrease in the chlorids of the blood, but this is merely temporary. In cases of marked nephritis, associated with retention of chlorids, the blood may show an excess of sodium chlorid.

As discussed on page 504, certain French workers have formulated a formula, as a working basis for a study of the excretion of urea in the urine. These same workers, especially Ambard and Weill, have applied this study to the excretion of sodium chlorid in human subjects. They have found that the same general laws, which were found applicable to urea, apply to sodium chlorid, with the exception that, while the excretion of urea occurs no matter how low its concentration in the blood plasma, there is a threshold for chlorid excretion, and, when the concentration in the plasma falls below this threshold value, excretion of chlorid practically ceases. In view of the fact that there is a wide difference in chlorid content of the corpuscles and plasma, plasma alone has been studied. The normal threshold value, as established by Ambard and Weill for chlorid, is 5.62 grams per liter of plasma. Hence the sodium chlorid above 5.62 grams per liter determines the rate of excretion, the law being expressed as for urea as follows:

Excess NaCl over 5.62 grams per liter of plasma

$$\sqrt{\frac{\text{NaCl in 24 hours}}{\text{Wt. in kilos}}} \sqrt{\text{NaCl per liter of urine}} = \text{Constant}$$

For practical use it appears best to calculate the plasma sodium chlorid from the rate of excretion, and to compare the calculated concentration with that actually found. The formula, as derived, with the use of values actually found for the constant in the above formula, reads

$$\text{Plasma NaCl} = 5.62 + \sqrt{D \times \frac{70}{\text{Wt.}}} \sqrt{\frac{C}{79.33}}$$

In which D equals grams of chlorid excreted in 24 hours; Wt. equals weight of the individual in kilograms; 70 equals the standard weight in kilos; C

¹ Bromberg (Jour. d'Urol., 1914, IV, 733) shows that the relation of the chlorids of the blood to those of the urine is normally 1 to 2 (hemo-renal index). Variations in this index are, he believes, the first indications of renal insufficiency. Gettler (Jour. Am. Med. Assoc., 1921, LXXVII, 1650) has introduced a method for determination of death by drowning, which depends on the estimation of the chlorid content of the right and left sides of the heart. A difference of the chlorid content of the two heart chambers exceeding 25 n. g. indicates that the individual was drowned. In cases of drowning in salt water, the chlorid content is larger in the blood of the left heart; while, in cases of drowning in fresh water, the right heart chamber shows the higher content. As it has been determined that no water can get into the left heart if the individual is thrown into the water after death, this test should have great medico-legal value.

equals grams of sodium chlorid per liter of urine. If this formula be simplified we obtain the following:

$$\text{Plasma NaCl} = 5.62 + \sqrt{\frac{D \sqrt{C}}{4.23 \text{ Wt.}}}$$

Assuming that the laws for rate of excretion of sodium chlorid over the threshold remain constant in normal individuals, one may calculate the threshold by subtracting the calculated excess from the sodium chlorid actually found in the plasma, giving us the formula,

$$\text{Threshold} = \text{Plasma NaCl} - \sqrt{\frac{D \sqrt{C}}{4.23 \times \text{Wt.}}}$$

This formula is subject to error if the rate of excretion over the threshold varies.

The method of collecting the specimens of blood and urine for the determination of the sodium chlorid threshold, as well as for the urea index according to McLean have been given on page 506, to which the reader is referred. After removing the portion of the whole blood for urea determination, the remainder is centrifuged at high speed to throw down the corpuscles and the plasma is pipetted off. The total chlorids of both plasma and urine are then determined, and calculated as NaCl. McLean¹ finds this threshold very constant at about 5.62 grams per liter, the actual average of his determinations being 5.61, the maximum variations being from 5.24 to 5.84 grams. The normal and usual range of concentration of chlorids in the human plasms is from 5.62 to 6.25 grams of NaCl per liter or higher, according to the amount ingested. The rate of excretion of chlorids gives a basis for calculating the theoretical concentration of chlorids in the plasma. By comparing the concentration actually found with the theoretical concentration, changes in the function of chlorid excretion may be studied. McLean² reports that relatively increased concentration of chlorids in the plasma occurs especially in certain forms of cardiac and renal disease; under certain conditions, notably in fevers or in diabetes, or as the action of diuretics, the chlorid threshold may be temporarily or permanently lowered; failure to excrete chlorids in pneumonia is associated with a lowered concentration of chlorids in the plasma, the excretion beginning at the time this concentration increases; edema is usually accompanied by a relatively increased concentration of chlorids in the plasma.

Determination of Plasma Chlorids.

Method of Van Slyke and Donleavy.

This method³ is a modification of the method of McLean and Van Slyke

¹ Jour. Exper. Med., 1915, XXII, 212.

² Ibid., 366. See, also, Rappleye, Boston Med. & Surg. Jour., 1920, CLXXXII, 89; Siebeck, Arch. f. exp. Path. u. Pharm., 1920, LXXXV, 214; Rusynak, Biochem. Ztschr., 1920, CX, 60; Host, Jour. Lab. & Clin. Med., 1920, V, 713; Fridericia, Jour. Biol. Chem., 1920, XLII, 245; Denis and Sisson, Ibid., 1921, XLVI, 483; Pruche, Presse Med., 1921, XXIX, 35; Killian, Jour. Lab. & Clin. Med., 1921, XII, 129.

³ Jour. Biol. Chem., 1919, XXXVII, 551; McLean and Van Slyke, Jour. Am. Chem. Soc., 1915, XXXVII, 1128; Jour. Biol. Chem., 1915, XXI, 361.

and has the advantage that the process may be simplified to a great extent and does away with the necessity of employing "Blood Charcoal" as a protein precipitant.

Solutions Necessary.

1. *Standard Silver Nitrate Solution.*—This is an acid M/29.25 solution of silver nitrate containing picric acid as the protein precipitant. 1 c.c. of this solution is equivalent to 2 mg. of NaCl. The composition is as follows: 5.812 grams of pure fused silver nitrate, 7.5 grams of purified picric acid, 250 c.c. of nitric acid (sp. g. 1.42), dissolved in sufficient water to make 1 liter. As a check on the accuracy of this solution, it may be standardized against a known hydrochloric acid solution by the Volhard method or gravimetrically.

2. *Standard Potassium Iodid Solution.*—This is a M/73.1 potassium iodid solution containing 2.27 grams of pure KI per liter. 1 c.c. of this solution is equivalent to 0.8 mg. NaCl, being so standardized that 2.5 c.c. is equivalent to 1 c.c. of the silver nitrate solution. This iodid solution is standardized against the silver nitrate solution as follows: 5 c.c. of the silver nitrate solution are accurately measured out and mixed with 5 c.c. of the starch-citrate solution (see below) and the iodid solution is run in from a buret to a permanent blue end-point. The amount required should be 12.65 c.c., 12.50 c.c. being required to precipitate the standard silver solution and 0.15 c.c. additional to give the end-point. In this standardization, as also in the test itself, only a permanent and unmistakable blue is taken as the end-point. If the iodid is run in rapidly toward the end of the titration, iodid may be formed more rapidly than the silver nitrate precipitates it, and a false end point reached. It is wise to make these titrations against a background of yellow paper. The amount of excess KI required to produce the end-point varies directly as the volume of the solution; consequently it is desirable to keep the volume at the end of the titration within approximately the same limits (25 to 30 c.c.) in standardizing as in performing the analyses.

3. *Indicator Solution.*—The composition of this solution is as follows: Sodium citrate 446 grams, sodium nitrite 20 grams, soluble starch 2.5 grams, water to 1000 c.c. The starch is first dissolved with the aid of heat in about 500 c.c. of water. The heating of this solution must be sufficient to accomplish the solution, hence the solution must be boiled for several minutes; if starch other than the soluble variety is used, the boiling should continue for an hour. The citrate and nitrite are then added and the heating continued until all is dissolved. Filter the solution through cotton, wash the filter with hot water, allow to cool and make up to 1 liter.

Technic.

Two c.c. of oxalate or citrate plasma are drawn into a dry pipet calibrated to contain 2 ± 0.005 c.c. The plasma is run into a 50 c.c. measuring flask half full of water, and the pipet is rinsed by drawing the water up into it twice. Ten c.c. of the standard silver nitrate solution are added and the

mixture is diluted to the 50 c.c. mark with water and shaken at intervals for several minutes, until coagulation is completed. The addition of a drop or two of caprylic alcohol prevents foaming and facilitates coagulation. The solution is now filtered through a dry, chlorid-free filter, the first portion of filtrate being passed through, if necessary, a second time to remove turbidity completely. Twenty c.c. duplicate portions of the filtrate are measured with a calibrated pipet into 100 c.c. Erlenmeyer flasks, 4 c.c. of the starch-citrate indicator solution are added to each, and the standard KI is run in from a buret until a permanent blue end-point is obtained.

Calculation.—This is very simple when standard solutions of the above concentration are used. The 20 c.c. of filtrate used for titration represent 0.8 c.c. of plasma, and the unprecipitated portion of an amount of silver nitrate equivalent to 8 mg. of NaCl, or 10 mg. per c.c. of plasma. Each c.c. of KI used in the titration is equivalent to 1 mg. of NaCl per c.c. of plasma. Hence the calculation simplifies to 10.15 c.c. of KI = mg. NaCl per c.c. of plasma or grams NaCl per liter of plasma. The use of 10.15 instead of 10 is due to the fact that 0.15 c.c. excess of KI solution is required to give the end-point. In this work it is necessary to check by calibration the accuracy of all pipets, burets, and measuring flasks used.¹

Method of Whitehorn.

This method² forms a supplement to the "System of Blood Analysis" of Folin and Wu and is employed with the blood filtrate, which is used for the other tests of this system. If one prefers, and this may seem advisable, the blood, after being drawn and oxalated in the usual manner, may be centrifuged and the plasma separated for the determination of the chlorid content.

The treatment of the blood or plasma is exactly the same as described under Non-protein Nitrogen of the Blood on page 497, water, sodium tungstate solution and sulphuric acid being added to precipitate the proteins.

Pipet 10 c.c. of the protein-free filtrate into a porcelain dish. Add with a pipet 5 c.c. of the standard silver nitrate solution³ and stir thoroughly. Add about 5 c.c. of concentrated nitric acid, and let stand for 5 minutes to permit the flocking out of the silver chlorid. Then add with a spatula about 0.3 gram of ferric ammonium sulphate and titrate the excess of silver

¹Other methods, or modifications of above, may be found as follows: Harding and Mason, *Jour. Biol. Chem.*, 1917, XXXI, 55; Foster, *Ibid.*, 483; Rappleye, *Ibid.*, 1918, XXXV, 509; Greenwald, *Ibid.*, 1919, XXXVIII, 439. For studies of the chlorids of the plasma see Frothingham, *Am. Jour. Med. Sc.*, 1915, CXLIX, 808; Christian, *Ibid.*, 1916, CLI, 630; O'Hare, *Arch. Int. Med.*, 1916, XVII, 711; Wolfierth, *Am. Jour. Med. Sc.*, 1917, CLIV, 84; Steinfeld, *Arch. Int. Med.*, 1919, XXIII, 511; Rodillon, *Presse Méd.*, 1920, XXVIII, 85; Myers and Short, *Jour. Biol. Chem.*, 1920, XLIV, 47; Wetmore, *Ibid.*, XLV, 113; Smith, *Ibid.*, 437; Austin and Van Slyke, *Ibid.*, 461; Myers, *Jour. Lab. & Clin. Med.*, 1920, VI, 17; Rieger, *Ibid.*, 44; 1921, VII, 166; Friend, *Jour. Biol. Chem.*, 1922, LI, 115; Iversen and Schierbeck, *Ugesk. f. Læger*, 1922, LXXXIV, 454; Iversen, *Ibid.*, 456; Isaacs, *Jour. Biol. Chem.*, 1922, LIII, 17.

²*Jour. Biol. Chem.*, 1921, XLV, 449.

³This is a M_{35.46} solution of silver nitrate. Dissolve 4.791 grams of C.P. Silver Nitrate in distilled water. Transfer this solution to a liter volumetric flask and make up to the mark with distilled water. Mix thoroughly and preserve in a brown bottle. One c.c. equals 1 mg. Cl.

nitrate with the standard sulphocyanate solution¹ until the definite salmon-red (not yellow) color of the ferric-sulphocyanate persists in spite of stirring for at least 15 seconds.

Calculation.

5.00 — titer (in c.c.) = mg. of Cl per c.c. of blood or plasma.

Since each c.c. of sulphocyanate solution used is equivalent to 1 c.c. of silver nitrate solution, the difference between the volume of silver nitrate solution taken and the excess determined by the titration, that is 5—titer, represents the volume which reacted with chlorid at the ratio of 1 c.c. to 1 mg. of Cl. And the 10 c.c. of filtrate taken represents c.c. of blood or plasma. To convert the figure for Cl into those for NaCl divide by 0.606.

Phosphates.

These compounds exist in the blood as neutral or alkaline salts of sodium, potassium, calcium, and magnesium, as well as in organic combinations in the red and white cells in the form of lecithin and nuclein. The inorganic phosphates are concerned, at least in part, with the solubility of the proteins and in maintaining the reaction of the blood through their "buffer" action. Just what variations in the amounts of the organic and inorganic phosphorus compounds occur in health and disease is unknown. It is certain that a definite phosphorus metabolism exists and that this is characterized, to a great extent, by variations in the normal relationship between the compounds of the alkali and alkali-earth groups. Just what these relations are in physiologic and pathologic states experiment must determine. According to Bloor, the inorganic phosphorus of the blood of normal individuals runs from 1.8 to 4.3 mg. per 100 c.c. of plasma, while Denis and Minot give these figures as 1.2 to 3.1 mg., and Tisdall as 3.5 to 4 mg. The latter states that the serum of normal infants has a much higher inorganic phosphorus content than is present in normal adult blood. In certain cases of nephritis Greenwald and Marriott and Howland have reported the presence of greatly increased amounts of inorganic phosphates in the plasma, while Denis and Minot show that about 65 per cent. of the nephritics and cardiorenal cases gave unmistakable evidence of phosphate retention; fatal cases show a rapid and progressive increase in the plasma phosphates. Howland and Kramer have demonstrated that the serum of infants suffering from active rickets contains a diminished amount of inorganic phosphates and that, following administration of cod liver oil, the phosphate content gradually rises to the normal level. Hess and Gutman have confirmed these findings and report the favorable effects of heliotherapy in increasing the phosphates. It is evident, therefore, that retention of phosphates may play some role in the possible diagnosis of nephritis and that, likewise, a decrease in this factor

¹ This is a M/35.46 solution of potassium or ammonium sulphocyanate. As the sulphocyanates are hygroscopic, it is not possible to weigh out accurately the exact amount required. Hence, these solutions must be prepared volumetrically. As an approximation about 3 grams of KCNS or 2.5 grams of NH₄CNS should be dissolved in a liter of distilled water. By titration under the conditions specified in the test and by proper dilution, a standard is prepared of such strength that 5 c.c. are equivalent to 5 c.c. of the silver nitrate solution. The method of making such dilutions is given in detail on page 212.

may be of some aid to the pediatrician.¹ That the system retains compounds of phosphorus more energetically than it does any other mineral constituents is proven. As the phosphates of the blood and of the urine come both from the food and from the breaking down of the nuclein-containing protein material of the system, large variations are possible under the influence of many factors. The subject of the metabolism of phosphorus is taken up under The Urine.

Method of Bell and Doisy.

This method² has been discussed under inorganic phosphates in urine on page 221, to which the reader is referred for composition and details of preparing the reagents used in this test.

Inorganic Phosphates.

Five c.c. of plasma are mixed with 15 c.c. of distilled water in a 25 c.c. volumetric flask and 5 c.c. of 20 per cent. trichloroacetic acid solution added with shaking. The flask is then filled to the mark with water, mixed, and, after 10 minutes, filtered through a phosphate-free (acid-washed) filter. If whole blood be used, 5 c.c. are laked by mixing with 35 to 40 c.c. of distilled water in a 50 c.c. volumetric flask and 5 c.c. of 20 per cent. trichloroacetic acid added with shaking. Make up to volume and filter after 10 minutes. If the plasma is suspected of being high in phosphate, a dilution of 1:10 may be used as in the case of whole blood. It has been shown by Denis and Meysenbug that certain specimens of exalated or citrated plasma either gave no color at all or such a faint one that color comparisons were impossible when treated with the various reagents used in the test. This result was due to the presence of an excess of oxalate or citrate in the plasma. For this reason, they advise that the determination of inorganic phosphate by this method be made with the serum, but where plasma must be used to restrict the amount of oxalate or citrate or, preferably, to increase the quantities of molybdic acid and hydroquinone. They recommend the use of 10 c.c. of the trichloroacetic filtrate, 2 c.c. of the molybdic acid solution and 2 c.c. of hydroquinone solution of double strength to that employed by Bell and Doisy (that is 40 grams per 1000 c.c.).

To 10 c.c. of the filtrate from above treatment in a 25 c.c. volumetric flask are added 1 c.c. of the molybdic acid solution, or as recommended by Denis and Meysenbug 2 c.c. (see urine) and 2 c.c. of the hydroquinone solution (of double strength). At the same time, molybdic acid and hydro-

¹ See Taylor and Miller, *Jour. Biol. Chem.*, 1914, XXVIII, 205; Greenwald, *Ibid.*, 1915, XXIX, 21; Marriett and Howland, *Arch. Int. Med.*, 1916, XVIII, 708; Feigl, *Biochem. Ztschr.*, 1917, LXXXI, 380; *Ibid.*, 1917, LXXXIII, 81; *Ibid.*, 1918, LXXXIV, 331; *Ibid.*, LXXXVI, 395; *Ibid.*, LXXXVII, 237; *Ibid.*, XCII, 1; Bloor, *Jour. Biol. Chem.*, 1918, XXXVI, 49; Feigl, *Biochem. Ztschr.*, 1919, XCIV, 293 and 304; *Ibid.*, 1920, CII, 131; Denis and Minot, *Arch. Int. Med.*, 1920, XXVI, 99; Bloor, *Jour. Biol. Chem.*, 1920, XLV, 171; McKollips, De Young and Bloor, *Ibid.*, 1921, XLVII, 53; Howland and Kramer, *Am. Jour. Dis. Child.*, 1921, XXII, 105; Hess and Gutman, *Jour. Am. Med. Assoc.*, 1922, LXXVIII, 29.

² *Jour. Biol. Chem.*, 1920, XLIV, 55. See Myers and Shevsky, *Jour. Lab. & Clin. Med.*, 1921, VII, 176; Hess and Gutman, *Jour. Am. Med. Assoc.*, 1922, LXXVIII, 29; Denis and Meysenbug, *Jour. Biol. Chem.*, 1922, LII, 1; Briggs, *Ibid.*, LIII, 13; Raudles and Knudson, *Ibid.*, 53.

quinone are added to a similar flask containing 10 c.c. (equivalent to 0.05 mg. of P.) of the dilute standard phosphate solution for blood (5 c.c. of the stock solution discussed under urine are made up to 1000 c.c. and preserved with chloroform. Five c.c. of this diluted solution contain 0.025 mg. P). As the standard contains no oxalate, it is unnecessary, of course, to change the quantities as suggested for the plasma filtrate. Add either 1 or 2 c.c. of 20 per cent. trichloroacetic acid solution, according to whether a dilution of 1:10 or 1:5 has been used. After 5 minutes, 10 c.c. of the carbonate-sulphite solution are added, and the flasks are made up to volume and mixed. The colors are compared after 5 to 10 minutes. If the unknown is read against the standard set at 20 mm., the calculation is

$$\frac{100}{\text{Reading}} = \text{mg. P. per 100 c.c. (if 1:10 dilution was used).}$$

or

$$\frac{50}{\text{Reading}} = \text{mg. P. per 100 c.c. (if 1:5 dilution was used).}$$

Total Acid—Soluble Phosphorus.

Ten c.c. of the trichloroacetic acid filtrate, in a hard glass test-tube, are evaporated down to about 2 c.c. with 6 to 8 drops of concentrated sulphuric acid and a piece of quartz. One c.c. of concentrated nitric acid is added and the digestion continued until all the nitric acid has been driven off and the remaining drops of sulphuric acid are clear. The residue is transferred to a 25 c.c. volumetric flask with about 10 c.c. of distilled water and treated as under inorganic phosphate. The standard to be used is determined by the value previously found for inorganic phosphate. Four to 6 drops of concentrated sulphuric acid are added to the standard to balance that used in the digestion, but no trichloroacetic acid is added. The comparisons are made in the same manner as for the inorganic P.

Method of Tisdall.¹

One c.c. of serum is transferred to a 15 c.c. centrifuge tube and to this are added 5 c.c. of a 6 per cent. solution of trichloroacetic acid. The mixture is thoroughly mixed with the aid of a glass rod and allowed to stand for 4 minutes. It is then centrifuged for 4 or 5 minutes at about 1500 revolutions per minute and the supernatant fluid poured off into another tube.

Five c.c. of this supernatant fluid are measured into an ordinary 15 c.c. graduated centrifuge tube, the outside diameter of which is 6 to 7 mm. at the 0.1 c.c. mark. Water is added to bring the volume to 6 c.c., followed by 2 c.c. of the strychnin molybdate reagent,² which should be added drop

¹ Jour. Biol. Chem., 1922, L, 329. For other methods see Marriott and Haessler, Jour. Biol. Chem., 1917, XXXII, 241; Bloor, *Ibid.*, 1918, XXXVI, 33; Iversen, Biochem. Ztschr., 1920, CIV, 22; Brauns and MaxLaughlin, Jour. Am. Chem. Soc., 1920, XLII, 2238; Embden, Ztschr. f. physiol. Chem., 1921, CXIII, 138; Wiener, Biochem. Ztschr., 1921, CXV, 42.

² Solution A is prepared by dissolving 50 grams of ammonium molybdate in 150 c.c. of warm water. If not clear this solution should be filtered. Solution B consists of 2 volumes of concentrated HNO₃ and 1 volume of water. Solution C is prepared by pouring 1 volume of Solution A into 3 volumes of Solution B. Solution D consists of a solution of 7.5 grams of strychnin nitrate in 500 c.c. of water. To prepare the reagent proceed as follows: 1 volume of Solution D is poured into 3 volumes of Solution C. The reagent should stand 24 hours before it is used. It will keep for at least 1 month. After the reagent has stood for 1 or 2 days, a slight precipitate forms and, when this occurs, it should be filtered off. Two c.c. of this reagent will precipitate 0.2 mg. of P.

by drop, and the tube shaken 3 or 4 times during the procedure. The contents of the tube are then thoroughly mixed by holding the tube at the upper end and tapping the lower end with the finger. The contents are allowed to stand for 10 minutes during which time they are thoroughly mixed twice as outlined above.

After the 10 minutes is up, the tube is centrifuged at 1500 revolutions per minute for 3 minutes, the supernatant fluid is poured off and the mouth of the tube wiped with a dry cloth. 3 c.c. of water are allowed to run down the sides of the tube, which removes any adherent supernatant fluid. The residual supernatant fluid is thoroughly mixed with the added water by tapping the lower end of the tube with the finger, while the precipitate is disturbed as little as possible. The mixture is centrifuged for 1 minute, the supernatant fluid is poured off and the above procedure repeated, making two washings in all.

After the final supernatant fluid has been removed 2 c.c. of a 1 per cent. solution of NaOH are added and the contents mixed with the aid of a glass rod. This causes all the precipitate to go into solution. Water is added to 10 c.c., and the contents are transferred to a 100 c.c. glass-stoppered volumetric flask. Traces of the solution remaining in the centrifuge tube are washed into the flask by means of two lots of 10 c.c. of water, making the total volume in the flask 30 c.c. Twenty c.c. of a 20 per cent. solution of potassium ferrocyanid are then added, followed by 10 c.c. of concentrated HCl. The flask is inverted two or three times and allowed to stand 10 minutes. Water is added to 100 c.c., the contents are thoroughly mixed, and the color is read in the colorimeter against the standard.

Standard Solution.

One c.c. of a solution of KH_2PO_4 containing 5 mg. of P. per 100 c.c. (219.3 mg. of the salt in 1000 c.c.) is measured into a graduated centrifuge tube, which contains 5 c.c. of water, and the contents are thoroughly mixed. Two c.c. of the strychnin molybdate reagent are then added drop by drop. This step, and the washing of the precipitate and the development of the color, are carried out at the same time and in the same manner with both standard and unknown. The amount of precipitate obtained in the standard solution after it is centrifuged is almost exactly 0.1 c.c. in volume. If the amount of precipitate obtained in the unknown is 0.2 c.c. or more, its solution (in 1 per cent. NaOH) should be made up to a definite volume in the centrifuge tube and an aliquot taken which would contain approximately 0.1 c.c. of precipitate. If the amount of the precipitate obtained in the unknown is about $\frac{1}{2}$ that in the standard, its solution should be made up to 5 c.c. and transferred to a 50 c.c. flask with the use of two lots of 5 c.c. of water. In all the subsequent steps the volumes used should be halved.

Calculation.

When the unknown is made up to 100 c.c. and the standard is set at 20 mm., the calculation is as follows:

$$\frac{20}{\text{Unknown}} \times 6 = \text{mg. of P. per 100 c.c. of serum.}$$

When the unknown is made up to 50 c.c., the result is divided by 2.

Calcium.

Calcium is beginning to have a position of more or less importance among the inorganic constituents of the body. It constitutes about $\frac{1}{50}$ of the body weight, which is a larger proportion than any of the other inorganic elements. However, it has been only recently that we were in a position to study this element in any other manner than by the urinary output. Thanks to the newer methods of micro-chemistry of the blood, we have been able to extend our knowledge of this important inorganic constituent to a certain extent. Various functions have been attributed to calcium, such as its obvious relation to the growth of the bones, its involvement in the process of clotting of the blood, its role in the maintenance of physiologic equilibrium, and its necessity for the proper maintenance of normal irritability of the nervous system, but the conditions under which it circulates in the blood and the amount in the blood, both in health and disease, have been more or less obscure.

It has been shown by the work of Kramer and Howland, with the more exact modern methods, that the concentration of calcium in the serum of normal adults is quite constant, about 9 to 10.5 mg. per 100 c.c. of blood. In normal children it is somewhat higher, running from 10.5 to 11.5. Whether there is any demonstrable amount of calcium in the corpuscles is still an unsettled question. This normal amount of calcium is difficultly changed by intake of calcium containing foods or of salts of calcium, as Denis and Minot have shown that the administration of 6 grams of calcium lactate orally each day for five days failed to alter the plasma content appreciably and Clark demonstrated that feeding a calcium-rich diet to animals had no effect on the calcium content of their blood. However, it is not to be definitely stated that the results, as shown by blood analysis, may not have some value in conditions of calcium deficit. Just how much calcium is needed by the body under normal conditions was somewhat uncertain until the work of Sherman, who has shown that the calcium requirement for the average normal adult of 70 kilos weight is 0.45 gram per day. In all probability the need is much greater in the growing child, whose bones are still in the process of development, but accurate figures of this amount do not seem to be available. In rickets, which is characterized by a deficient deposition of calcium salts in the bones and in the epiphyseal cartilages, it might be supposed that there was a calcium deficit in the blood, but Howland and Kramer find that, during the period of active rickets, the calcium concentration of the serum may be normal or slightly reduced, while Maysenbug and McCann have shown that the diffusible calcium in the serum of rachitic subjects is between 60 and 70 per cent. of the total serum calcium, a finding which is identical with that obtained by them for normal individuals. On the other hand, Denis and Talbot report that a low calcium content of the serum frequently occurs in acute rickets. In rickets it has been found that there is a marked reduction of the inorganic phosphorus of the blood, which probably accounts for the deficiency in calcium phosphate for bone formation. Aside from this important field of rickets, it would seem that there are only

two conditions, which have been as yet investigated, in which there is any marked reduction in the calcium content of the serum, namely, in tetany and nephritis. Just how much importance is to be attached to this finding in nephritis remains to be determined, but there can be little question of its value in tetany.¹

Method of Kramer and Tisdall.

One or 2 c.c. of serum are measured into an ordinary 15 c.c. graduated centrifuge tube containing 2 c.c. of water. 1 c.c. of a saturated solution of ammonium oxalate is then added and the sample mixed. The mixture is allowed to stand for $\frac{1}{2}$ hour. The volume is then made up to 6 c.c. with distilled water and the sample again mixed. The tube is centrifuged at 1300 revolutions per minute for 10 minutes. All but 0.3 c.c. of the supernatant fluid is siphoned off and saved for the determination of magnesium. The precipitate is mixed with the remaining fluid and 2 per cent. ammonia (2 c.c. of concentrated ammonia to 98 c.c. of water) is added up to 4 c.c. and mixed. The tube is then centrifuged for 5 minutes. All but 0.3 c.c. of the supernatant fluid is again siphoned off. The last step is repeated twice, making 3 washings in all. The tube is centrifuged for 5 minutes after each washing. After the last washing the supernatant fluid is siphoned off. The crystals, which remain in the centrifuge tube are suspended in the residual liquid and dissolved in 2 c.c. of approximately normal sulphuric acid, heated in the water bath for a few minutes, and then titrated to a definite pink color, which persists for at least 1 minute, with 0.01 N potassium permanganate. This is delivered from a micro-buret graduated in 0.02 c.c.

Calculation.

The number of c.c. of 0.01 N potassium permanganate used—the blank (volume of 0.01 N potassium permanganate required to produce the same intensity of color in an equal volume of water) $\times 0.2$ = the number of mg. of calcium in the serum taken.

The only reagent that must be quantitatively accurate is the 0.01 N sodium oxalate, which must be used for the standardization of the potassium permanganate. The 0.1 N sodium oxalate is prepared in the usual way, 6.7 grams of dry, chemically pure salt being dissolved in 1 liter of water. The solution of the oxalate is facilitated by the addition of 5 c.c. of concentrated H_2SO_4 . This solution is then diluted 10 times to make the 0.01 N sodium oxalate solution.

The first supernatant fluid removed from the precipitate of calcium oxalate may be used for determination of magnesium should this be desired.

¹ See Howland and Marriott, *Quart. Jour. Med.*, 1913, XI, 296; Denis and Minot, *Jour. Biol. Chem.*, 1920, XLI, 357; Handovsky, *Jahrb. f. Kinderhkde.*, 1920, XCI, 432; Stheeman and Arntzenius, *Nederl. Tijdschr. v. Geneesk.*, 1920, I, 1030 and 1168; Underhill, Honeij and Bogert, *Jour. Exp. Med.*, 1920, XXXII, 41 and 65; Krehbiel, *Jour. Cancer Res.*, 1920, XIV, 237; Jacobowitz, *Jahrb. f. Kinderhkde.*, 1920, XCII, 256; Clark, *Jour. Biol. Chem.*, 1920, XLIII, 80; Sherman, *Ibid.*, XLIV, 21; Jones and Nye, *Ibid.*, 1921, XLVII, 321; von Mysenbug and McCann, *Ibid.*, 541; Jones, *Ibid.*, XLIX, 187; Denis and Talbot, *Am. Jour. Dis. Child.*, 1921, XXI, 29; von Mysenbug, *Ibid.*, 150; Howland and Kramer, *Ibid.*, 1921, XXII, 105; Kramer, Tisdall and Howland, *Ibid.*, 560; Richter-Quittner, *Wien. Arch. inn. Med.*, 1921, II, 217; Blatherwick and Long, *Jour. Biol. Chem.*, 1922, LII, 125.

However, little is known regarding the variations or the significance of magnesium in the blood, so that this portion of this test will be omitted at the present time.¹

Iron.

The iron of the blood is found principally in the hemoglobin which contains about 0.42 per cent. of Fe. It is also found in traces in the plasma, and, according to Hammarsten, in the nuclein compounds. The attempt to estimate the amount of hemoglobin by determinations of the blood iron have proven failures, as no definite relations exist between them. All of the blood iron is not in the form of colored compounds and, moreover, some of the derivatives of hemoglobin are iron-free. Biernacki has shown that the direct quantitative estimation of iron yields higher results than could be obtained by computation from the percentage of hemoglobin, this finding being confirmed by Jolles and Jellinek.

According to Schmidt and Becquerel and Rodier, the amount of iron in the blood ranges between 0.056 and 0.058 per cent. while Berman's² figures range from 0.0402 to 0.0559 per cent. Female blood shows a somewhat lower value, just as it does for hemoglobin. For the estimation of the the iron of the blood, I must refer to other works for the details, as the method is too cumbersome for general clinical use. The principle of the method is based on the comparison of the colors of a known solution of iron treated with ammonium sulphocyanate solution, with that of a solution of blood iron, obtained by incinerating blood, fusing the ash with potassium bisulphate, dissolving the fused mass in water, and treating it with sulphocyanate solution. The instrument used for the colorimetric tests is called Jolles' ferrometer, the amount of iron in 1,000 c.c. of blood being obtained by reference to a table accompanying the instrument. If it is desired to obtain the actual percentage by weight, the calculation must include the determination of the specific gravity of the blood. Having this latter factor, we may use the equation $G : V :: 100 : X$, in which G represents the specific gravity and V the percent-

¹ Jour. Biol. Chem., 1921, XLVII, 475. For other tests for calcium see Howland, Haessler and Marriott, Jour. Biol. Chem., 1916, XXIV., proc. XVIII; Laws and Cowie, Am. Jour. Dis. Child., 1917, XIII, 236; Lyman, Jour. Biol. Chem., 1917, XXIX, 160; Ibid., XXX, 1; Halverson and Bergeim, Ibid., XXXII, 159; Halverson, Mohler, and Bergeim, Jour. Am. Med. Assoc., 1917, LXVIII, 1309; Jour. Biol. Chem., 1917, XXXII, 171; Marriott and Howland, Ibid., 233; Cowie and Calhoun, Ibid., 1919, XXXVII, 505; Kramer and Howland, Ibid., 1920, XLIII, 35; Kramer and Tisdall, Bull. Johns Hopk. Hosp., 1921, XXXII, 44; von Meysenbug, Pappenheimer, Zucker and Murray, Jour. Biol. Chem., 1921, XLVII, 529; Clark, Ibid., XLIX, 487; Kahn and Hadjopoulos, Proc. Soc. Exp. Biol. & Med., 1921, XVIII, 203; Ling and Buohill, Biochem. Jour., 1922 XVI, 403. For tests covering Ca, Mg, Na and K see Greenwald, Jour. Pharm. & Exp. Therap., 1918, XI, 281; Greenwald and Gross, Proc. Soc. Exp. Biol. & Therap., 1919, XVII, 50; Kramer and Tisdall, Jour. Biol. Chem., 1921, XLVIII, 223; Hammett and Adams, Ibid., 1922, LII, 211; Briggs, Ibid., 349; Denis, Ibid., 411. For tests for Sodium in blood, aside from those last cited, see Kramer, Jour. Biol. Chem., 1920, XLI, 263; Doisy, and Bell Ibid., 1921, XLV, 313; Kramer and Tisdall, Ibid., 1921, XLVI, 467; Wilson, Ibid., 1922, L, 301. For Potassium see Kramer, Ibid., 1921, XLVI, 339; Myers and Short, Ibid., XLVIII, 83.

² Jour. Biol. Chem., 1918, XXXV, 231. See Brown, Jour. Am. Chem., Soc., 1922, XLIV, 423.

age by volume of iron which is obtained from the table. The hemoglobin value may be found by use of the formula of von Jaksch, $Hb = \frac{100 \times M}{0.42}$, in which M equals the percentage of iron by weight.

(J) Blood Gases.

The gases existing in the blood are oxygen, carbon dioxid and nitrogen, the latter having little importance in the body functions, its amount corresponding to that which would be absorbed by an equal volume of distilled water, namely, 1.8 volumes per cent.

The amount of oxygen and of CO₂ varies widely, depending on the arterial or venous character of the blood, upon the velocity of the blood flow, upon the temperature, amount of exercise, etc. Oxygen occurs principally in chemical combination with hemoglobin, but a small amount, about one-fourth per cent., is present in solution in the plasma.¹ About one-tenth of the CO₂ is held in solution in the blood, while the remaining nine-tenths is apportioned as follows, one-third loosely bound to the alkalies and hemoglobin of the corpuscles, two-thirds held in chemical combination with the alkalies and proteins of the plasma. The transport of CO₂ in the blood is chiefly carried out by the corpuscles.

The following table made up from the figures of Setschenow, Ludwig and Sczelkow, and Hammarsten, will show the relation between these gases.

	<i>Arterial.</i>	<i>Venous.</i>
Oxygen,	21.6% by volume.	6.8% by volume.
Carbon dioxid,	40.3% by volume.	48.0% by volume.
Nitrogen,	1.8% by volume.	1.8% by volume.

These figures are somewhat at variance with those given by Lundsgaard and by Harrop. The former states that the maximum oxygen content of normal human venous blood is 17.98 volumes per cent., the minimum value is 9.55, and the average is 13.6. The difference between the total oxygen-combining power of the hemoglobin and the oxygen in the venous blood is termed the "oxygen unsaturation" of the venous blood, the average figure thus being 5.8 volumes per cent. Harrop shows that the oxygen content of the arterial blood ranges from 13.89 volumes per cent. to 24.08, with a percentage saturation of 94.3 to 100; the oxygen content of venous blood varies from 10.52 to 17.61 volumes per cent.; the carbon dioxid content of arterial blood ranges from 44.58 to 54.69 volumes per cent., while that of venous blood varies from 48.27 to 60.43 volumes per cent. These figures vary, of course, with conditions which interfere with the proper exchange of gases in the lungs and on the influence of the carbon dioxid of the blood on the respiratory center, as well as upon the power of the blood to take up carbon dioxid through the agency of the buffer salts of the plasma.²

¹ See Peabody (Jour. Exper. Med., 1913, XVIII, 7) for a discussion of the oxygen content in pneumonia; also, Preti, Riforma Med., 1915, XXXI, 432, for variations in blood gases in uremia.

² See Van Slyke, Jour. Biol. Chem., 1918, XXXIII, 127; Lundsgaard, Ibid., 133; Jour. Exp. Med., 1918, XXVII, 179, 199 and 219; 1919, XXX, 147; Harrop. Ibid., 241; Straub and Meier, Biochem. Ztschr., 1920, CIX, 47; Poulton, Jour. Physiol., 1920, LIII, LXI;

(K) Ferments of the Blood.

The fact has been well established by Jacobi that the various organs and tissues of the body contain ferments which are proteolytic as far as the corresponding tissues are concerned, but are usually inactive when applied to the proteins of other organs.¹ In other words, these ferments are autolytic, but not, as a rule, heterolytic. Much experimental work of former and latter years has shown that many metabolic processes, associated with the building up and breaking down of various tissue elements, are influenced to a great extent by the presence of ferments arising from many sources.

It is not unreasonable to assume that the blood, like other tissues, contains various ferments which have to do with general and special metabolism. The work of Schönbein on the oxidases of the blood, of Hanriot and, indirectly, of Castle and Loevenhart on the lipolytic ferment, of Lepine, Seegen, and Spitzer on the glycolytic ferment shows that such properties are resident in the blood. The work of Ascoli and Moreschi, Jochman and Müller, and of Stern and Eppenstein has opened up an entirely new field of work on the proteolytic properties of the leucocytes. This proteolytic ferment action is both autolytic and heterolytic and is influenced to a great extent by the checking action of the antiferments, which have been so well studied by Opie. Further, it has been shown that differences exist between the proteolytic properties of the many varieties of leucocytes and that the ferments are not always heterolytic (Mosse).

It would lead me too far afield to discuss this subject in detail; hence, I must be content with reference to the presence of these substances.² Much benefit will be forthcoming from a further study of such properties of the blood and of certain constituents of the blood. It is to be recalled that ferment action may be accountable for the influence of the toxophore and other groups of Ehrlich's complement, but this phase must be discussed later.

(7) Enumeration of Red and White Cells.

This section of hematological technic is, perhaps, the most perfected and most frequently employed. The red and white cells are usually counted, as the enumeration of the platelets has little practical or scientific value at the

Haldane, *Jour. Path. & Bact.* 1920, XXIII, 445; Meakins and Davies, *Ibid.*, 451; Krogh, *Biochem. Jour.*, 1920, XIV, 267; Parsons, *Ibid.*, 1921, XV, 202; Barseh and Woodwell, *Arch. Int. Med.*, 1921, XXVIII, 367, 394 and 421; Van Slyke, *Physiol. Reviews*, 1921, I, 141; Smith, Means and Woodwell, *Jour. Biol. Chem.*, 1921, XLV, 243; Newcomer, *Ibid.*, 1921, XLVII, 489; Van Slyke and Stadie, *Ibid.*, XLIX, 1; Stadie, *Ibid.*, 43.

See Menten (*Jour. Med. Res.*, 1919, XI, 433) for a study of general oxidase reactions.

² Smithies (*Jour. Am. Med. Assn.*, 1912, LIX, 539) has called attention to the presence in human blood-serum of an agent (probably a ferment) which shows peptid-splitting properties. See Caro (*Ztschr. f. klin. Med.*, 1913, LXXXVIII, 286), who finds a lipolytic ferment in the serum. Also, Neumann, *Biochem. Ztschr.*, 1913, L, 347; Zamorani, *Pediatrics*, 1914, XXIII, 401; Porter, *Munch. med. Wehnschr.*, 1914, LXI, 1775; Resch, *Deutsch. Arch. f. klin. Med.*, 1915, CXVIII, 179; Satta, *Arch. Ital. Biol.*, 1915, LXIV, 118; Sagal, *Jour. Med. Res.*, 1916, XXXIV, 231; Killian and Myers, *Proc. Soc. Exper. Biol. and Med.*, 1916, XIV, 32; Myers and Killian, *Jour. Biol. Chem.*, 1917, XXIX, 179; Sloan, *Am. Jour. Physiol.*, 1917, XLII, 558; De Niord and Schreiner, *Arch. Int. Med.*, 1919, XXIII, 484; Burge, *Jour. Lab. and Clin. Med.*, 1919, V, 59; Fujimoto, *Am. Jour. Physiol.*, 1919, L, 208; Caro, *Ztschr. f. klin. Med.*, 1920, LXXXIX, 49; Euler and Borgenstam, *Biochem. Ztschr.*, 1920, CII, 124; Bach and Zoubkoff, *C. R. Acad. des Sc.*, 1920, CLXXI, 667; Krumbhaar and Musser, *Jour. Am. Med. Assoc.*, 1920, LXXV, 104; Lewis and Mason, *Jour. Biol. Chem.*, 1920, XLIV, 455; Morgulis, *Ibid.*, 1921, XLVII, 341; Am. *Jour. Physiol.*, 1921, LVII, 125; Karsner, Koechert and Wahl, *Jour. Exp. Med.*, 1921, XXXIV, 349; Welker, *Jour. Lab. & Clin. Med.*, 1921, VII, 173.

present time and the latter technic is not sufficiently perfected to admit of conclusions.

Various instruments have been introduced for the purpose of counting the corpuscles in a given volume of blood. Most of them are based on the principle that a layer of diluted blood, of a certain depth, covering a certain known space, shows a definite number of corpuscles for every drop of blood used. The general method of making the count consists in diluting the fresh blood in definite proportions with some indifferent fluid and counting, under the microscope, the number of cells in a drop of this diluted blood, which is contained in a small glass cell on the floor of which is ruled a series of micrometer squares of known dimensions. The cubic contents of the cell and the degree of dilution of the blood being known, the number of corpuscles counted in any given number of squares of the ruled area may be taken as a basis for calculating the total number of cells in a cmm. of blood. Strong and Seligmann dispense with a special counting chamber and enumerate the cells in a definite quantity of blood diluted in exact proportions with a diluent stain and mounted as a permanent specimen. Einhorn and Laporte use a somewhat similar method and arrive at very good comparative results.

The normal number of red cells in the adult male is, approximately, 5,000,000 per cmm. of blood, while in the female it is somewhat lower, namely, 4,500,000. Marked variations in this figure are observed in pathological conditions and will be discussed in a later section along with the treatment of the physiological factors which influence the number and appearance of these erythrocytes. In the normal adult the number of white cells varies between 5,000 and 10,000 the average being 7,500. This figure is subject to both physiologic and pathologic influences to a greater extent than are the red cells.

Many instruments, such as those of Hayem, Gowers, Malassez, Thoma-Zeiss, Alferow, and Durham, have been introduced, but the most universally used and the best adapted for such investigation is, in the writer's opinion, that of Thoma-Zeiss. This combines certain modifications of the mixing pipet of Malassez, the counting chamber of Hayem, and the micrometer rulings of Gowers. It gives us, therefore, a most complete instrument for such work.

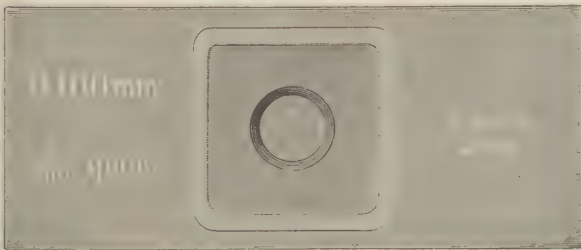


FIG. 132.—Thoma-Zeiss counting chamber.

Hemocytometer of Thoma-Zeiss.

The blood counter, originated by Thoma and constructed by Zeiss, is all that could be desired. Leitz, Reichert, and other makers of optical

goods have introduced similar types of counters, but our experience with them has not been so good. So much depends upon the accuracy with which pipets are graduated and upon the trueness of the rulings of the counting chamber that we recommend the general use of the Zeiss instrument.

This instrument consists of a pipet for mixing the blood to a certain dilution, a counting chamber by means of which a layer of known depth and area is obtained, and a special cover-glass for the chamber.

Pipets.

The original form of the Thoma apparatus included but one pipet this being used for the dilution when both red and white cells were to be counted. Experience has shown, however, that the dilution given by this pipet is, in most cases, too great to permit of accurate counting of both types of cell.¹ A modification has, therefore, been introduced to allow of greater accuracy by giving a lower dilution and by furnishing a larger number of cells in the counting chamber.



FIG. 133.—Diluting pipets: Erythrocytometer; leucocytometer with Croy's attachment.

Erythrocytometer.

This is the original pipet of Thoma. It consists of a graduated capillary tube (A) opening into a dilatation (B), at the opposite end of which is a shorter, glass tube (C), graduated with a line marked 101 and to which is attached a rubber tube with an ivory mouth-piece. This pipet is so graduated that the capacity of the ampulla (B) is exactly 100 times that of the capillary tube from its point to the line marked 1 and 200 times that from the point to the line marked 0.5. Other lines, both below and above this latter point, are calibrated on the tube, each line representing one-tenth of the capacity of the capillary. In some of the pipets, especially that of the Miescher hemoglobinometer, two smaller marks each representing one one-hundredth of the length of the tube are calibrated on each side of the major divisions. By this means the dilution of the blood may be definitely known if the tube be not filled exactly to the point described. Those pipets with obtuse ends are much to be preferred to those with the more acute angles. In the ampulla is a small glass bead which is of service in properly mixing the blood with the diluting fluid. This pipet may be used in counting both the red and white cells and is all that is absolutely necessary when one uses the Zappert or Türk counting chamber. In general work, however, it is used only in the count of the red cells as the extreme dilution given is better adapted to this estimation than it is to the counting of the white cells.

¹ See Ellermann, *Deutsch. Arch. f. klin. Med.*, 1913, CIX, 378.

Leucocytometer.

This is a graduated capillary tube similar to the erythrocytometer in construction but having a larger lumen, which will permit of lower dilutions. It is graduated into ten divisions, with the marks 0.5 and 1 representing these measures of the total capacity of the capillary. Above the ampulla is a graduation, 11, which is so calibrated that blood, drawn to the mark 1 and diluted to 11, gives a dilution of 10, while if drawn to the point 0.5 the dilution is 20. Those forms of this pipet which have the lower end tapering to a fine point giving a gradually decreasing lumen, are much to be preferred to the older models. As the caliber of this instrument is relatively large, the student is cautioned against using too great suction in making the dilution, and also against placing too large a drop on the counting surface.¹

The Counting Chamber.

"This consists of a heavy glass slide, A, on which is cemented a thick glass ring, B, the surface of which is highly polished. This ring surrounds

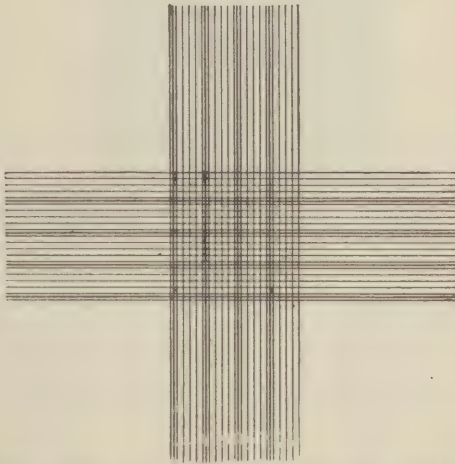


FIG. 134.—Ruled surface of Thoma-Zeiss counting chamber. (*Da Costa*.)

a circular table of glass, D, the height of which is just 0.1 mm. less than that of the surrounding ring. Between this glass table and the inner edge of the ring is a small ditch, C, to catch the drop which may run off from the table and to prevent its flowing up between the ring and the cover-glass on the other side of the ditch. On the central glass table cross at right angles 21 parallel lines, equidistant, and between the extremes of which is exactly 1 mm. Hence we have an area of 1 square millimeter divided into four hundred small equal squares. Through each fifth row of squares

¹ My colleague, Dr. C. C. Croy, has devised a most excellent and useful pipet attachment (see above cut), which prevents leakage of the contents during transportation. This consists of a strong rubber band with two disks of any kind of thin metal folded on the rubber in such a way that the ends of the pipet come in contact with the rubber and press against the metal disks. See, also, Saxon and Drummond, *Jour. Am. Med. Assn.*, 1915, LXV, 1182; Moody, *Ibid.*, 1921, LXXVII, 941.

is ruled an extra line, which is not a boundary but is merely an aid in keeping one's position in the ruled area. Indicated, not bounded, by these extra lines, the square millimeter is divided into 16 units of 25 small squares each" (Emerson).

As the ruled surface of one square millimeter is divided into 400 small squares, each small square has an area of $\frac{1}{400}$ square mm. The height of the column of blood being $\frac{1}{10}$ mm., the cubic contents of each small square is $\frac{1}{4000}$ cmm.

This counting chamber of Thoma does not permit of the counting of a sufficiently large number of leucocytes, especially when the dilution has been made in the same degree as is used for the enumeration of the red cells. In order to overcome this difficulty and also to give a larger ruled area in which

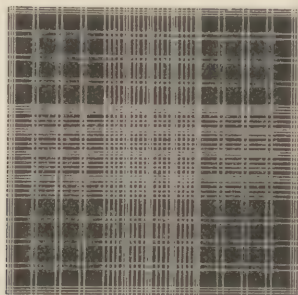


FIG. 135.—Turk's ruling of the counting chamber.

the leucocytes may be counted, Zappert has modified the original ruling in such a way that a counting surface of 9 sq. mm. is afforded. This modification has been improved by Ewing and by Türk in such a way that the four large corner squares, each of 1 sq. mm., are subdivided into 16 smaller squares, each of which is equal in area to the total 25 smallest squares of the Thoma chamber. This latter modification is much the best and is used exclusively by the writer. Its advantage in counting both red and white cells will be appreciated when the student compares it with the older chamber. The sixteen central squares are used in counting the erythrocytes, while the entire area may be used in the enumeration of the leucocytes. Simon has recently introduced a different modification of the Thoma ruling, which is extremely simple and should prove very satisfactory. Other forms have been introduced by Bürker, Gorjajew and Pappenheim and, more recently, by Bass. All give excellent results.

Before use the counting chamber should be well washed with water and carefully dried. Precautions should be taken to see that no lint is left on the surface of the glass ring and that no alcohol or ether are used in the cleaning process, as these substances loosen the cement with which the glass table is fastened to the slide.

The Cover-glass.

This is made of heavy polished glass with accurately planed surfaces. The ordinary cover-glasses should never be employed with the counting-chamber as they are often uneven in surface and do not fit tightly to the slide. Moreover, these ordinary cover slips are so thin that the capillarity of the drop of blood may bend them down to some extent. The cover-glass must be as carefully cleaned and dried as is the chamber.

Diluting Fluids.

In order that the blood may be properly examined, it must be diluted with a solution which will at the same time prevent coagulation and hemolysis and will preserve the corpuscles intact. There are numerous formulæ for such solutions and the choice is largely a matter of experience. Much will depend on whether the red and white cells are both to be counted at the same dilution or whether two different pipets are used in making the dilutions. Personally the writer prefers the use of two pipets using different diluents, but other workers use one pipet and one diluent. The red cells may be destroyed by certain fluids leaving the white cells intact or the white cells may be colored by the same diluent used in counting the reds.

Hayem's Solution.

This solution preserves the red cells permanently and permits the corpuscles to settle slowly, thus furnishing an even distribution of the cells. Moreover, it will keep almost indefinitely and does not permit of the development of yeast spores which so readily multiply in many of the other diluents. The writer can recommend this diluent as the most generally useful of the preparations advised. It is made up as follows:

Mercuric chlorid,	0.500 gram.
Sodium sulphate,	5.000 grams.
Sodium chlorid,	1.000 gram.
Distilled water,	200.000 c.c.

Owing to the presence of mercuric chlorid, this fluid cannot be mixed with an aniline coloring substance to stain the leucocytes and is, therefore, not applicable to the combined counting of the red and white cells.¹

Toisson's Fluid.

Sodium chlorid,	1.000 gram.
Sodium sulphate,	8.000 grams.
Neutral glycerin,	30.000 c.c.
Distilled water,	160.000 c.c.
Methyl violet 5 B.,	0.025 gram.

The addition of methyl violet serves to color the leucocytes and permits of their recognition along with the erythrocytes. Occasionally this fluid

¹ Jörgensen (Ztschr. f. klin. Med., 1914, LXXX, 21) believes the results with this solution are much improved by diminishing the mercuric chlorid content to 0.1 gram (one-half part per liter).

hemolyzes the red cells and thus invalidates the count. Moreover, it easily becomes infected with yeast spores which develop profusely in it. For this reason it is advisable to filter the fluid before use, each filtration, however, weakening it, so that it becomes after a time useless.

Other diluents, such as the solutions of Pacini, Löwit, Petrone, Acquisto, Edington, and Callison have been used, but the more generally applicable ones are those mentioned above. Toisson's fluid is particularly useful when the count of reds and whites is to be made in the same specimen. The coloring of the leucocytes is not necessary for their recognition, but it is a convenience to one who is not making blood counts frequently. Hayem's solution is the very best diluent at our command for general purposes.

If it is desired to count the white cells alone, and this is always wise, a 1 per cent. solution of acetic acid, to which is added gentian violet to bring out the white cells a little more clearly, may be used. This solution destroys the red cells and thus gives only the white cells in the preparation. For this reason the addition of the gentian violet is unnecessary. Yeast cells develop in this solution with more or less readiness, hence one should employ only freshly made solutions, as these yeast cells resemble, to some extent, mononuclear leucocytes and may introduce an error into the count.¹

Method of Counting the Corpuscles.

With this process of counting the cells, whether red or white, there are five steps to be taken:

1. **Obtaining the blood.**
2. **Diluting and mixing the blood.**
3. **Filling the counting chamber.**
4. **Counting the cells.**
5. **Cleaning the apparatus.**

Erythrocytes.

(1) Obtaining the Blood.

As previously stated, the blood may be drawn from a puncture of the ear or finger. Personally, the writer always uses the ear, unless some valid reason exists for not doing so. As soon as a good-sized drop appears, which is obtained without pressure or constriction, the tip of the pipet is placed in the drop and is supported by a finger of the left hand, which holds the ear in position. The blood is drawn by suction to the mark 0.5, in cases in which anemia is not suspected, or to the mark 1 in such cases, as a routine the former mark being preferable. As the student will find, some practice is needed to stop the column of blood exactly at the point desired. If the blood be drawn a little too far, the tip of the pipet may be rubbed with the finger or the excess may be shaken down by tapping the tip against a towel or, preferably, the blood may be drawn to the next mark and the necessary correction made in the dilution. Unless this error can be corrected, the pipet must be cleaned or a second one used. In any event, if the blood be not accurately measured,

¹ Seilin (Jour. A. M. A., 1916, LXVII, 1387) calls attention to the fact that a trace of copper sulphate added to this solution prevents the development of molds and yeasts.

we must reject the determination. Moreover, the work should be done rapidly to prevent coagulation of the blood. Hence, too much time should not be spent in adjusting the column of blood.¹

(2) Diluting and Mixing the Blood.

As soon as the column of blood is adjusted at the desired height of the capillary, the tip of the pipet is carefully wiped with the fingers to remove any adherent blood and is immediately dipped into the diluting fluid in such a way that no portion of the blood is lost. Türk recommends closing the end of the pipet with the finger and exerting slight suction on the closed tube in order to prevent any loss on immersion of the pipet. I have never seen it necessary to use this measure, providing ordinary care is employed in applying the suction as soon as the pipet touches the diluent. The diluting fluid should stand ready in a small dish or bottle and should be carefully examined before use to see that no flocculi or spores are present.

The diluent should be at once drawn into the capillary by suction. The fluid rises slowly in the tube, the pipet which is held vertically being rotated between the finger and thumb of the left hand as the fluid rises. By this rotation, the diluting fluid is mixed with the blood at once and bubbles of air, which often cling to the inside of the tube, are avoided. The glass bead in the ampulla serves the purpose of thoroughly mixing the blood and diluent. As the column approaches the mark 101 on the upper end of the pipet, care should be taken that the aspiration is not too strong. While the error, introduced by drawing the diluted blood beyond this mark, is not as great as it is in drawing the undiluted blood to the mark 0.5, yet both should be avoided. When this upper mark is reached, withdraw the pipet from the diluent, close the tip with the finger, bend the rubber tubing down over the other end, and close this with the thumb. Some prefer to remove the rubber tubing at this juncture, but this is not at all necessary. Shake the pipet vigorously for at least one minute to insure a thoroughly uniform mixing of the contents. If for any reason the count is not to be made at once or if it be desired to carry the blood to the laboratory for examination, the rubber tubing may be removed and the ends of the pipet closed by means of a rubber band. Naturally, before the mixture can be used it must be again thoroughly shaken. If the examination is to be done at once, proceed as follows. Blow three or four drops from the pipet in order to remove the column of fluid which has remained in the capillary and has not mixed with the blood. If Toisson's fluid has been used as the diluent, it is better, before blowing out the drops, to allow the pipet to lie horizontally for 10 to 15 minutes in order to permit of the staining of the leucocytes.

(3) Filling the Counting Chamber.

As a rule, it is better, unless Toisson's fluid has been the diluent, to fill the counting chamber at once as errors may creep in by allowing the pipet

¹ See Maddox (Jour. Am. Med. Assn., 1913, LX, 663) for the description of a new device aiding in filling the pipets. Yarbrough (Jour. Lab. & Clin. Med., 1921, VII, 172) uses oxalated blood and takes it in this form to the laboratory, claiming that the counts are as accurate as those made at once.

to stand, even though the later mixing may be thorough. A small drop, the size of which can be learned only by experience, is blown onto the center of the ruled area of the counting chamber. The drop should not be so large as to run over into the moat, but should be large enough practically to cover the glass table.

Adjust the cover-glass at once. This is a point in the technic which requires considerable practice and must be mastered before accurate results can be obtained. Emerson's advice on this point is admirable, "grasp the cover-glass by two diagonal corners, place a third corner against the slide with the edge of the glass ring as a fulcrum, and hold it in that position by a finger of the left hand. By now raising the finger the cover is rotated onto the drop rapidly and also in such a way that no air-bubble is left." Breathing upon the cover-glass before it is adjusted is often serviceable in making this preparation. If there has been no dust on the slide or cover-glass and they are perfectly clean, the student will observe, providing the adjustment has been properly made, a beautiful band of colors known as Newton's rings, which are due to a phenomenon of interference of light. If these rings do not appear, they may often be brought out by firm pressure on the edges of the cover-glass. If they are not persistent, after the pressure has been removed, the adjustment of the two polished surfaces must be assumed to be imperfect and the preparation rejected.¹ It happens at times that these rings are difficult to obtain and some workers state that they are not necessary for an accurate count. The writer prefers, however, to reject those slides which do not show these diffraction rings than to run the risk of including a possible error. These rings may best be seen by holding the slide on a level with the eyes in such a way that the light is totally reflected from the surface of the cover-glass. After the slide is properly adjusted allow it to stand for three or four minutes before proceeding with the next step of the determination, in order to insure the proper settling of the corpuscles upon the counting table.

(4) Counting the Cells.

Before any count of the cells is attempted, the entire surface covered by the blood must be examined with the low-power lens to ascertain whether the distribution of the cells is uniform throughout. If not, the slide should be rejected, even though the points mentioned previously have obtained. It is much better to stop proceedings at this point than to attempt to equalize an uneven distribution by a larger counting area. If the specimen proves satisfactory the count may then be undertaken. The lenses best suited to this purpose are Bausch & Lomb $\frac{1}{6}$, Zeiss D, Leitz 6 or 7, and Reichert $\frac{1}{6}$. As the student becomes accustomed to cell-counting he may use a lower objective. This has the advantage of bringing the entire field of one sq. mm. into focus. A mechanical stage is of some convenience in making the count, but

¹ Eustis (Jour. Am. Med. Assn., 1913, LXI, 1084) advises the following: Grasp the cover-glass between thumb and index-finger of right hand. Hold the slide firmly on the table with the left hand and rapidly slide the cover-glass across the counting chamber, moving the glass in a perfectly horizontal manner. See, also, Hartz, New York Med. Jour., 1915, CL, 612; Lyon (Jour. A. M. A., 1917, LXVIII, 709) calls attention to possible inaccuracies in the size of the counting chamber and pipets.

the fingers answer quite as well after some practice in manipulating the slide.

The unit of counting surface is a matter of individual preference. Sahli recommends a unit of four small squares, Grawitz and Simon use sixteen of these squares, while Cabot advises the use of 36. In common with Türk, Ewing, Da Costa, Wood, and Emerson, the writer prefers the units of 25 small squares, as this is the unit of the ruling and the calculation is much simpler than with the other units.

In order to simplify the process of counting, some routine method must be used. These methods depend on the worker, but the usual procedure in the writer's laboratory is as follows: Adjust the slide upon the table of the microscope so that the upper left-hand corner of the central ruled area of 16 large squares of the Türk chamber is brought into the field in such a manner that one may count the cells in the small squares from left to right. If the low-power objective is used, the complete group of 16 units will lie within the field of observation and may be easily examined. With the higher power, which the student may be forced to use in his earlier work, the optical field is necessarily limited. Count the total number of cells lying within the 25 small squares of the single unit. In doing this count the upper row of the unit from left to right, drop down a row, and count the cells from right to left and so on until the cells in the whole unit have been counted. The accompanying cut will indicate the method to be followed. In making this count, cells which touch the right hand or lower boundaries of the unit are disregarded, while those which touch the upper and left-hand line are included in the count of that square. After counting the cells in the upper left-hand unit, count those in the remaining fifteen units, thus covering a field of 16 units of 25 small squares each, making a total of 400 small squares counted. Türk recommends the counting of eight units as a minimum, Emerson advises the counting of the four corner units in each of two separate preparations, while Da Costa and others count the cells in four groups of units from above downward and repeat with four units not adjacent. It seems to the writer that the error is less the larger the area covered, and he, therefore, advises the beginner to make the total count of the sixteen units, although it is not supposed that one will try to cover up defects in technic by this larger counting area. A variation of more than 25 cells in the counts of the various units should be taken as evidence that the distribution is not perfect. In such cases the count would better be rejected on the ground of inaccuracy. After the technic is mastered and the worker has discovered just exactly wherein his error lies, a count of 8 or 4 units will suffice. It is advisable, where accurate scientific results are indispensable, to clean the slide and make a second count with a fresh drop of blood so that one may have a check on his work. For clinical purposes, however, the count of four units will ordinarily be sufficient.

In ordinary counting it is not necessary to differentiate the red cells from the leucocytes, as the error thus introduced is small and may be disregarded. In cases, however, which show large leucocyte values, this error will be quite

appreciable and must be overcome by the following method. All of the cells observed may be counted as erythrocytes and the reduction made for the number of leucocytes as obtained by the special leucocyte count to be described later. In the use of Hayem's diluting fluid, the leucocytes are not colored, while the erythrocytes retain their normal yellow color. The leucocytes appear, by good illumination of the field, of a bluish tone, are somewhat larger than the red cells, and are characterized by a sharper border. These facts will enable one after some experience to distinguish the white from the red cells.

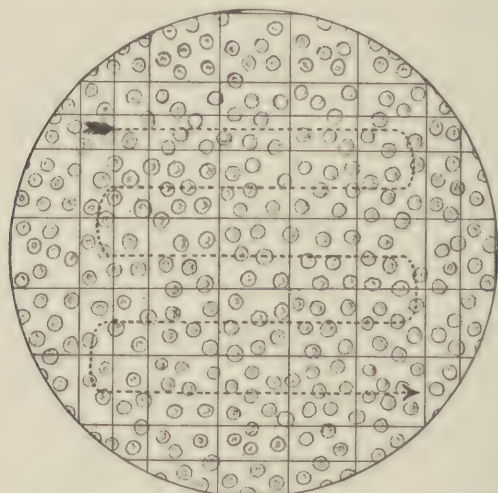


FIG. 136.—Plan of Counting the Cells. (DaCosta.)

The small squares are examined in the order indicated by the arrow.

Toisson's fluid stains the leucocytes blue, and may, therefore, be used to outline these cells. As a rule, it is better to learn to recognize the leucocytes by differences in refraction than to rely on the staining qualities of these cells. In some cases the methyl violet of the diluent colors some of the red cells so that they cannot be easily distinguished from the white ones.

The number of cells in the blood is invariably reported as the number contained in a cubic millimeter of blood. In making the calculation of this number it is necessary to know the number of units counted, the number of cells in these units, the area of each small square of the unit, and the degree of dilution of the blood. Thus, if 16 units, each of 25 squares, have been counted, the total number of small squares is 400, each having a cubic area of $\frac{1}{4,000}$ mm. The total volume of the units counted is, therefore, $\frac{1}{10}$ mm. It is evident that the number of cells in 1 mm. of blood is 10 times that in the area counted over if the blood were undiluted. But the count is always made with diluted blood and we must, therefore, take this factor into account. With a dilution of 100 multiply the number of cells in 1 mm. by 100, and with a dilution of 200 multiply by this factor. Thus, if 2,500 cells were counted in the 400 small squares and the cubic contents of the units gone over

was $\frac{1}{10}$ cmm. then 1 cmm. of diluted blood would contain 25,000 cells. As the blood was diluted 200 times, the total number of cells in one cmm. of undiluted blood is 5,000,000. A very simple way of remembering this calculation is to multiply the number of cells counted in the total area of 400 small squares by 1,000 if the dilution was 100, and by 2,000 if the dilution was 200.

If the total area of 16 units be not counted, the method of calculation is the same but the factors are variable. This method goes as follows: Multiply the number of cells counted by the degree of dilution and this result by the cubic contents of each small square (4,000). Divide this result by the number of small squares counted. Thus, to calculate the number of cells in a cmm. of blood when 100 small squares (4 units) were counted at a dilution of 200, the count being 625 cells, the equation is as follows:

$$= 5,000,000. \quad \frac{625 \times 200 \times 4000}{100}$$

Leucocytes.

In counting the leucocytes much depends on the sort of ruled slide at the disposal of the worker. With the old Thoma chamber at least five different drops must be examined in order that a sufficiently large number of leucocytes may be counted, while with the Türk cell a counting area of 9 sq. mm. is afforded for each drop. The more leucocytes counted so much the less is the error. It is usually sufficient to count the white cells in a single drop, using the Türk chamber, but for scientific purposes three or even four drops would better be examined.

If it is desired to count the leucocytes in the same specimen as the red cells the procedure is as follows: Prepare the drop of blood exactly as described for counting the red cells, using Toisson's fluid as the diluent and the erythrocytometer as the diluting pipet. After the reds have been counted, enumerate the whites in the entire ruled area of this chamber. In this process the leucocytes will be found to be stained a faint blue. It is often advisable, in case of low leucocyte counts, to repeat this process with a second drop of blood. The calculation by this method is very simple. As the entire ruled area of the Türk chamber covers a surface of 9 sq. mm., each equal to the central area used in counting the red cells, we have the equivalent of 3,600 small squares in the ruled surface. Multiply this figure by the number of drops used to obtain the total number of small squares covered by the count. Thus, if 54 leucocytes were observed in two drops (7,200 small squares) and the dilution was 200, then we have the equation,

$$\frac{54 \times 200 \times 4000}{7200} = 6,000 \text{ cells.}$$

A second method of calculating the number is to consider each sq. mm. of the surface of the Türk chamber as a unit. If then, the number of cells counted in two drops (18 units) be 54, we divide this number by the number of units counted, 18, and multiply the result by 10 (the cubic contents of each unit) and then by the dilution. Thus,

$$\frac{54 \times 10 \times 200}{18} = 6,000.$$

It is usually preferable in counting the leucocytes to use the special leucocytometer previously described, as this gives a smaller dilution, and consequently a larger number of leucocytes to the counting surface. In using this pipet, the blood is drawn to the mark 1 and the diluent, 1 per cent. acetic acid, added to the mark 11. This diluent destroys the red cells and brings out the leucocytes clearly. The dilution of the blood will thus be 10. If a large increase in the number of leucocytes is anticipated, it is better to use a dilution of 20, drawing the blood to the mark 0.5, as a routine the dilution of 10 being, however, preferred.

In counting the white cells with a dilution of 10, using the Türk chamber, it is not as a rule necessary to go over the entire counting field, but more accurate results will obtain if this be done. A count of at least 250 cells is advisable, while one of 1,000 is more to be preferred in scientific work. The method of calculation is the same as given above. Thus if 540 cells were counted in the 9 sq. mm. of the ruled surface, the dilution being 10, we have

$$\frac{540 \times 10 \times 10}{9} = 6,000. \text{ If we have not used the total 9 sq. mm., but have}$$

used 4 or any other number which may be considered sufficient by the worker, the divisor in this division will be the number of units counted.

If only a Thoma chamber be at hand for counting the cells, two methods are available. By the first, several drops may be gone over counting the total number of cells in the ruled sq. mm. of the cell. In the second method we find the cubical contents of each visual field and then count the leucocytes in many of such fields. The method of computing these factors is given by Stengel¹ to whose work the author refers the reader who may not have access to a Zappert or a Türk chamber.

In making this leucocyte count great care must be exercised to have the diluent fresh and free from yeast spores, which so freely develop in such mixtures. If this factor be observed, all the cells seen may be counted as leucocytes, but occasionally nucleated red cells may be confusing, especially if these be present in large numbers. The physiological condition of the patient should always be considered in making a report on a leucocyte count, as such factors as digestion and exercise influence this count to a great extent.

The normal error in making a leucocyte count, with a count of 200 and more leucocytes, is about 5 per cent., while in the case of a red count it should not be over 3 per cent. Careful work with special attention to all the details mentioned will often reduce this error to a lower figure. Naturally, the error in counting the leucocytes will be much reduced by using the Türk chamber and giving the blood a dilution of 10. There are certain errors due to faulty construction both of the pipet and of the counting chamber which remain constant in the same apparatus. Hence it is wise to procure the very best equipment possible and to test the different portions of the pipet for such errors. In this way a very appreciable difference may be obviated. Not all

¹ Twentieth Century Practice of Medicine, New York, 1896, VII, 271.

of these blood counters show such variations, but some do, and it is, therefore, a matter of moment to know your tools.

(5) Cleaning the Apparatus.

This is the last step in the technic of making a blood count. While perhaps not as important as some of the other steps, yet if not properly carried out it will introduce errors which may prove very annoying. It is readily seen that an unclean pipet or counting chamber will interfere with the proper manipulations as described above.

After the cover-glass has been removed from the slide, wash out the chamber with distilled water and dry thoroughly by means of a clean piece of linen. Place the slide in its proper receptacle, so that it may be conveniently found when desired. This may seem a small point, but the author has seen

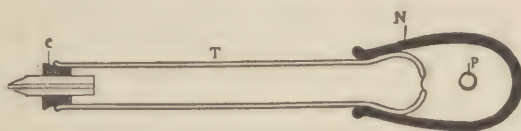


FIG. 137.—Cross-section of Durham's blood pipet. (*Da Costa.*)

T, Glass tube; N, rubber nipple; p, lateral perforation in nipple; c, cork in which a capillary pipet is fitted.

too many slides lost by carelessness in putting them away. The cover-glass is rubbed clean and dry and put in the case with the slide.

Wash out the pipet with water until all of the blood is gone. In some cases fine clots will be observed sticking to the side of the tube. Under such conditions remove the adherent blood by means of a fine wire or, if this is not effective, draw a little strong potassium hydrate solution into the capillary and allow it to act on the clot. After the tube is apparently clean, wash it out with alcohol and ether. Blow a stream of air through the tube by means of compressed air or the suction pump. Be certain that the apparatus is perfectly dry and clean, and that the glass bead in the ampulla is freely movable as the tube is shaken, before putting the pipet in its case.

Durham's Hemocytometer.

Recently Durham has introduced a modification of the older instruments for blood-counting. This embodies the principles of the various methods, but substitutes a self-measuring capillary pipet for the suction pipet of the Thoma apparatus, and special mixing vessels for the dilution of the blood. This device makes it possible for one inexperienced in blood-counting accurately to measure the blood and diluting fluid and thus eliminates the error possible with the older pipet. The direct count is made in the counting chamber of Thoma. This capillary of Durham is more easily cleaned than that of Thoma, thus giving an advantage in cases where several blood examinations are to be made in a limited time. While this method of diluting the blood has the advantages above mentioned, the writer has never been able to convince himself that the use of this apparatus gives the student any better results than he obtains with the Thoma instrument. After some

experience, and this is needed in any method, the dilution of the blood with the Thoma pipet is quite as easily made as with the Durham modification, and the results of the count give quite as close checks as do those with the newer method.

Oliver's Hemocytometer.

This instrument was intended to furnish a more accurate method of counting the red cells than was given by the older instruments. The method is based on a principle entirely different from that of the older instruments and

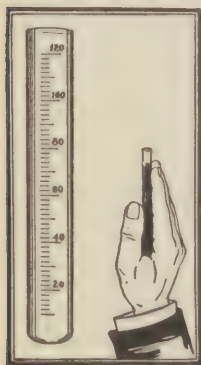


FIG. 138.—Oliver's hemocytometer. (Greene.)

does not afford an actual count of the cells. If blood be diluted with a fluid, which preserves the corpuscles, in a rectangular test vessel composed of longitudinally striated glass, each striation of the glass will act as a lens projecting an image of a candle flame viewed through the suspension of opaque particles of the blood, providing the suspension is of a sufficient dilution to permit of the almost unobstructed passage of the rays of light. At the proper dilution, these images of the candle flame will form a bright streak horizontally across the tube. Experiments have shown that the development of this bright line, on dilution of the blood with Hayem's solution, is an accurate measure of the percentage of red cells in the specimen examined. The dilution of the blood is made in

a rectangular glass cylinder by means of a capillary pipet, which is washed out with Hayem's solution. The cylinder is graduated into divisions from 10 to 120, each division representing 50,000 red corpuscles.

As this instrument has little clinical value, owing to the fact that the error is very great in cases in which the blood is diseased, I refer the reader to other works for a description of the method in detail. In the study of the physiologic variations of the red cells, this method affords very accurate results, giving figures which would be lost with the Thoma instrument. It must be remembered that, in this method, as in many others the personal equation plays a large rôle and may account for serious error which is not found with the Thoma instrument. Emerson has shown that the variations in the results may be as much as 2,000,000 cells when this instrument is compared with the Thoma in counting the blood in primary anemia. Baumgarten has proven that variations in the size of the cell as well as deformities of the cell will introduce a serious error into this method. Ellermann and Erlandsen¹ as well as Dunger² have recently introduced new methods, for making accurate counts of the white cells, which may prove very advantageous.

Counting of the Blood-platelets.

The technic of counting these cellular elements has been imperfect and the results variable. The methods advanced are both direct and indirect. The technic of Determann and of Brodie and Russell belong to the latter class,

¹ Deutsch. Arch. f. klin. Med., 1910, XCVIII, 245.

² Münch. med. Wchnschr., 1911, LVIII, 1131.

PLATE XVII.



FRESH NORMAL BLOOD. (ZEISS OCULAR 4, OBJECTIVE DD.)

while the method of Helber is a direct one. Recently *Wright and Kinnicutt*¹ have introduced a method which is simple, exact and reliable. The technic is as follows: The blood is diluted 1:100 by means of the pipet used for counting the red cells and the counting is done in the Thoma-Zeiss counting-chamber, using all the precautions previously discussed. The specially thin cover-glass of Zeiss, with central excavation, is used to render the platelets clearly visible. The diluting fluid consists of 2 parts of a 1:300 aqueous solution of "brilliant cresyl blue" and 3 parts of a 1:1400 aqueous solution of potassium cyanid. These two solutions should be fairly fresh, kept separate, mixed and filtered just before taking the blood. After the counting chamber is filled, it is left at rest for 10 to 15 minutes in order to allow the blood-platelets to settle to the bottom of the chamber and be more easily and accurately counted. The platelets appear as sharply outlined, round, oval or elongated lilac-colored bodies, some of which form a part of small spheres or globules of hyaline substance. The red cells are decolorized and appear as "shadows," while the nuclei of the leucocytes are stained a dark blue and their protoplasm light blue. This method shows a normal platelet count of 225,000 to 350,000 per cu. mm. No constant relations seem to obtain between the variations in the number of platelets and of the leucocytes. According to Determann, the ratio between the red cells and the blood plates is, on the average, 22:1. The pathologic variations in the number of these cellular elements will be discussed in detail in a later section.

III. MORPHOLOGY OF THE BLOOD

Before any examination which is concerned with the study of the morphological characteristics of the blood can be made, it is essential that all of the glassware which comes in contact with the blood should be absolutely clean and dry. The glass slides, as they come from the shops, are often coated with substances which are removable with difficulty. Moreover, these slides are not in all cases perfectly level on both surfaces. It will need but one experience with an uneven slide to convince the worker that it is a loss of time to attempt the use of such slightly convex or concave slides. The cover-glasses should be of the very best quality of glass, should be as thin as possible (number 0), and three-fourths inch square. The seven-eighths inch square covers as also the larger rectangular ones are not as desirable for blood work, especially in the examination of fresh specimens.

The slides and covers should be cleaned with soap and water followed by water and alcohol. In some cases it is necessary to soak them in concentrated hydrochloric acid for some hours and then wash with water, alcohol, and ether, or the ordinary acid-alcohol may be used. After being cleaned they should be kept either in 95 per cent. alcohol or, preferably, polished with a clean linen cloth or a piece of tissue-paper and kept in dust-proof receptacles. It is a wise precaution invariably to polish the slides and covers before use, as dust particles are prone to collect even under the best conditions. As a

¹ Jour. Am. Med. Assn., 1911, LVI, 1457. See, also, Fonio, Deutsch. Ztschr. f. Chir., 1912, CXVII, 176; Thomsen, Hospitalstid., 1919, LXII, 161; Acta Medica Scandinav., 1920, LIII, 507; Gram, Ibid., LIV, 1; Arch. Int. Med., 1920, XXV, 325; Buckman and Hallisey, Jour. Am. Med. Assoc., 1921, LXXVI, 427; Herwerden, Ibid., 723.

rule, it is better to use only new cover-glasses and not attempt to clean them after use. The slides may, however, be cleaned by boiling with a strong alkali solution, washing with hot water acidified with hydrochloric acid, then with hot water, alcohol, and ether.

After the cover-glasses have been polished it is the best practice to handle them only with forceps, as moisture is almost certain to collect on them if the fingers be used. This is not only better technic, but more rapid work may be done with their use. Two kinds of forceps are necessary in such work. The first is one for holding the cover firmly, being found as the locking forceps of Ehrlich or the cross-point forceps, while the second is the ordinary pinch forceps with which the second cover-glass is handled in making smears.

(1) Examination of Fresh Blood.

The examination of fresh blood is a very important part of hematological work and should be a routine procedure in every case possible. If the blood cannot be examined for several hours after being taken, it is wise not to attempt the study of a fresh specimen, as so many changes will occur in such slides that no certain findings obtain. The information obtainable from such examination of fresh blood often supplements that which one may derive from a study of the stained specimens, as some peculiarities, such as the ameboid movement of the leucocytes or the motility of the malarial parasite, may be studied only in this way.

Technic.

Assuming that the slides and cover-glasses are clean and dry, the ear is punctured as previously described. Wipe away the first few drops of blood and touch the center of a cover-glass, held with the pinch forceps, to the top of the next drop, which should be about the size of a small black-headed pin. If this drop be too large the layer of blood will be too thick to permit of proper examination. Care should be taken that the cover-glass does not touch the skin. Drop this cover onto a slide, which has been warmed by rubbing or by passing through a flame. If the glassware be clean, the drop will spread evenly in a thin circular layer, not quite to the edge of the cover-glass. Under no circumstances should pressure be used to thin the layer or to readjust the cover after it has settled on the slide, as artefacts may be easily introduced in this way. The slides thus prepared are examined first with a low-power lens to obtain an idea of the even distribution of the cells over the entire area. The detailed study is carried out with the $\frac{1}{12}$ oil immersion lens, but it should be remembered that a smaller magnification may give a better general survey. These preparations will keep long enough for the purposes of examination, but if one wishes to preserve the blood fresh and uncoagulated for a longer period it is well to enclose the cover-glass with vaselin or paraffin or to use the ordinary hanging-drop chamber as suggested by Rosin and Bibergeil. It is sometimes desirable, especially in the study of malarial parasites, to use a warm stage or a warm chamber. If, however, the specimen is examined soon after its preparation, no such precaution is necessary, provided the room is not cold.

In order to judge of the changes which abnormal blood may show in the fresh state, one must be thoroughly familiar with the appearance of normal blood. This latter knowledge can be obtained only by frequent study of fresh normal specimens and not from any text-book description. To attempt to learn without microscopic study the size, shape, color, and refraction of the red and white cells, the relation of the blood-plates to fibrin formation, the number of the various cells and their ratio to one another, would be absolute idiocy.

An examination of the fresh blood as described above gives information regarding the presence or absence of the malarial parasite, the spirochete of relapsing fever, the filaria, and trypanosomes. It affords evidence of increased or decreased rouleaux formation, number, deformities, and degenerations, as well as of the amount of hemoglobin of the red cells; the presence of a leucocytosis or of a leucopenia and of ameboid movement of the leucocytes. However, care must be taken to avoid premature conclusions from such study and to institute further examinations of the stained specimen to clear up doubtful points. The observer must be on his guard lest he mistake the normal Brownian movement in the protoplasm of the cells for ameboid or parasitic movement. Curious phenomena are observed in the fresh specimen as the blood dries and should not be misinterpreted. The various characteristics of fresh blood will be taken up in detail later. The introduction of the ultra-condenser or dark-field illuminator has opened up a field of great possibilities in the examination of specimens of fresh blood, especially when malarial parasites or spirochætæ pallidæ are suspected.

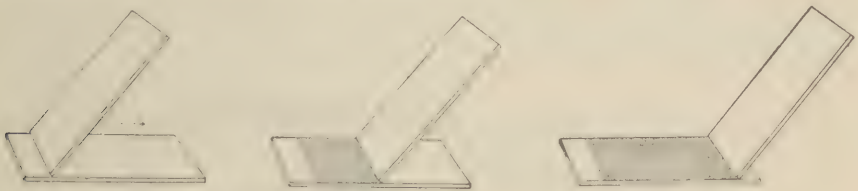


FIG. 139.—Preparation of smears with two glass slides. (*Da Costa.*)

(2) Preparation of Smears.

To prepare blood smears, which are to be later examined in the stained condition, one may spread the blood in capillary layers on slides or between cover-glasses. The former method is the one used in the writer's laboratory and has given excellent and satisfactory results. A fair-sized drop of blood is collected on one end of a clean dry slide, held between the thumb and second and third finger of the left hand. A second slide is held in the same manner by the right hand, but at an angle of 45 degrees to the first one and touching the drop of blood. Allow the blood to spread out by capillarity along the edge of the second slide. As soon as this occurs, draw the drop of blood along the first slide with a clean sweep, exerting little pressure with the second slide and maintaining the angle of 45° between the two slides, allowing the second slide to rest rather upon the blood than upon the slide (see cut). In the process, as recommended by some writers, the second slide is gradually drawn

into a position perpendicular to the first one. This procedure does not yield, in the writer's hands, as good results as the former method, as it is more difficult to maintain equal pressure, the smear being as a result too thick or too thin in places. Instead of a slide, a cigarette paper may be used as a spreader and gives good results. This method of making blood smears has the advantage of offering a large surface for examination, of making smears which are fairly uniform after some practice, of dispensing with the necessity of mounting the specimen, and of permitting the fixation of the smear in the

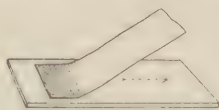


FIG. 140.—Preparation of blood smear with cigarette paper. (*Da Costa.*)

free flame. It is less expensive than the method to be described later and permits of the cleaning and later use of the slides. The beginner may find, on examining his early specimens made by this method, that the leucocytes collect at the distal end of the smear and that the general surface contains few white cells. This is due to the use of undue pressure in making the smear, and may be avoided by proper attention to this detail.

A second method which has many advocates is the use of two cover-glasses. One clean, dry cover-glass, which should not be too large (preferably three-fourths inch square or the larger rectangular slips), is held by the Ehrlich or cross-bladed forceps or, as some advise, between the thumb and first finger of the left hand. The other cover, held in the pinch forceps or between the thumb and first finger of the right hand, is touched to the drop of

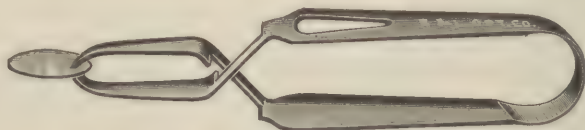


FIG. 141.—Ehrlich forceps.

blood as it wells from the puncture in the ear. This second cover is then dropped at once upon the first in such a way that the corners of the two glasses do not coincide. If the glasses are clean the blood spreads out evenly in a thin capillary layer between them. As soon as the spreading is complete, the two covers are drawn apart, in a line parallel to the plane of their surfaces, by a steady, quick motion, being sure to avoid lifting them apart. This manipulation can be learned only by practice and never from any description. If the fingers are dry and if care be taken to touch only the corners of the covers the forceps need not be used in separating the covers, but it must be remembered that moisture will cause changes in the specimen. It is, therefore, advisable to use the forceps in this part of the technic unless the rectangular slips be used. As soon as the covers are separated, they are allowed to dry in the air or by waving them two or three times to and fro. They should be at

once placed in a clean, closed receptacle, such as a Petri dish, until ready for the later complete fixation and staining, as dust will collect upon them or flies may attack them if left in the open air. It is rarely necessary to fix these smears at once, but with some stains such treatment is advisable. This second method of making the blood smears is more difficult than the first, is not so reliable, does not give as great a surface for examination, and always shows the lower cover-glass better and more uniformly spread than the upper.

Smears made by either of these methods should be uniform throughout with the exception of the edges, which should never be used as they are too thick for allowing definite conclusions to be drawn. The red cells should lie on their broad surface, should not be in rouleaux except at the edges, and should not show deformities due to errors in technic. The leucocytes frequently collect at the edges of the specimen if too great pressure be used in

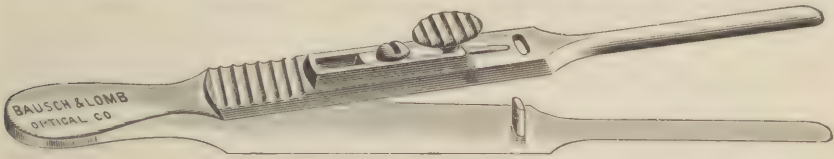


FIG. 142.—Pinch forceps.

making the smear, while the platelets always collect at the point first touched by the second slide or cover-glass. The preparation of thin even smears is necessary for the proper carrying out of the later technic. Those specimens which are irregular or are too thick would better be discarded, as the time consumed in studying such specimens will not be compensated for by the results obtained. It is much better to make several smears than to be content with a few bad ones. A little experience with poor smears will convince the worker that it is advisable to use great care in preparing them, especially if a differential count is to be made or if a study of the degenerations and deformities of the cells is to be undertaken.¹

(3) Fixation of Smears.

Before any staining of the cellular elements of the smear takes place, the protein constituents of the blood must be coagulated by exposing the air-dried film to the action of a high degree of heat or to that of various chemical reagents. The selection of the method of fixation will depend to a great extent upon the stain to be used later. Fixation is always essential if aqueous stains are used, while it is not so necessary if strong alcoholic solutions are employed. In the use of the different modifications of the Romanowsky stain, the fixation is done by the methyl alcohol employed as a solvent for the various stains. As a general rule, fixation by heat is preferable to that by chemicals, as artefacts are less prone to appear, providing the degree of heat is carefully regulated.

Fixation by Heat.

This method, which is the most difficult to use and which is at the same time the best, is the only one which is reliable when Ehrlich's triple stain is to

¹ See Schilling, *Deutsch. med. Wchnschr.*, 1913, XXXIX, 1985.

be employed. The principle is as follows: The air-dried specimen is subjected to the action of a temperature of 110° to 150° , for a more or less varying length of time, depending on the experience of the special worker. The lower the temperature the longer must its action be exerted.

The apparatus most frequently employed is the copper plate introduced by Ehrlich. This is an unpolished triangular plate of copper about 3 mm. thick, 30 to 50 cm. long, and 10 cm. wide, which is held in position by vertical standards. It is heated by an alcohol lamp or a Bunsen burner placed under the narrower end until the temperature of the plate becomes constant, the parts nearer the flame being naturally warmer than those more remote. The temperature of the different portions of the plate may be readily found by determining the points at which water (100), toluol (110), xylol (140), or of turpentine (150) boil. The slides or cover-glasses are then placed, smeared side up, at the desired point (the outer margin of the glass being three-fourths inch from the boiling-point and toward the flame). Just how long a period is necessary, at the temperature selected, to give a perfect fixation will depend

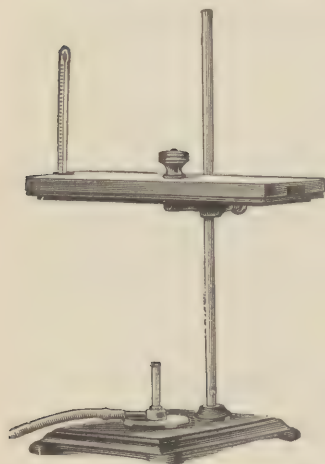


FIG. 143.—Oven for fixing blood films. (*Da Costa.*)

upon the age of the specimen and upon the condition to be studied. It is good practice to place several slides at the point desired for a period of one hour and then remove a slide at intervals of 15 minutes thereafter. One of the specimens is sure to be good by this method and the remaining ones may, therefore, be properly heated. Freshly made specimens require longer heating than the old ones, while normal blood requires a longer exposure than abnormal specimens. As a rule, the specimens require, when the triple stain is to be used (and this is the one most frequently employed with heat fixation), from one to one and one-half hours at a temperature of 110° , although some workers use only a few minutes' (one to three) exposure to such temperatures. The higher the tempera-

ture the less time is essential. Rubinstein uses a point at which a drop of water does not boil, but assumes the spheroidal state (so-called Leidenfrost phenomenon) and places the slides, with the smeared side down, upon the plate at this point for one-half to three-fourths minute. Some workers, as Pappenheim, use this point, but place the smeared side upward.

Instead of the copper plate, one may use the ordinary drying oven or a Victor Meyer heater. It is the writer's custom to use a copper drying oven heated by a gas flame regulated by a thermostat. The slides, with the smeared side downward, are placed on a glass plate whose temperature is measured by an accurate thermometer. The temperature is allowed to increase gradually to about 80° , from which point it is more quickly raised until the desired stage is approximated, when the heating must continue slowly, the

final temperature being maintained for 15 minutes. The best temperature for fixation, in the writer's experience, is 110° to 120° for 15 minutes when the staining is to be done with eosin-methylene blue or eosin-hemotoxylin, while for the tri-acid stain a temperature of 120° to 125° for one and one-half hours should be maintained. Some workers heat to 160° rapidly and then allow the films to cool to 30° , when fixation is complete in about 15 minutes. Engel and Cabot recommend, in the absence of other equipment, the passing of the smear through the flame several times. Such treatment often yields excellent results, but is uncertain and requires much experience. It is to be remembered that too rapid changes of temperature are to be avoided, as shrinking or splitting of the cells will occur under such conditions. So much depends upon proper fixation that a little more time spent in obtaining good specimens will shorten the time necessary for the future examination. Proper staining depends on proper fixation, especially with the tri-acid stain, in which cases the over- or under-heating is evident in the color tone of the erythrocytes.

Chemical Fixation.

(1) Absolute Alcohol.

Allow the alcohol to act for five minutes to one hour, or boiling alcohol for one minute. The slide is simply covered with the fixative and left for the desired period. Much depends upon the stain to be used as to the time necessary for good fixation. If an alcoholic stain, five minutes is sufficient; if a watery or alkaline one, a longer time is essential. After fixation drain off the alcohol and allow the residue to evaporate in the air or wash with water and dry between sheets of filter-paper. If alcoholic stains are to be used the moist specimen may be directly passed through the flame. This fixative is unreliable if a study of the neutrophile granules is to be made, but it brings out the nuclear structures fairly well.

(2) Nikiforoff's Method.

Cover the smear with equal parts of absolute alcohol and ether and allow the fixative to act from one-half to two hours. After the fixation allow the fixative to evaporate or wash with water and dry. Some workers advocate short fixation especially where the malarial organism is to be studied. This fixative brings out the degenerations of the red cells in good shape.

(3) Methyl Alcohol.

This fixative is used absolutely pure for three to five minutes. It is the most generally applicable chemical fixative and gives beautiful specimens. If used in combination with stains, as in the Romanowsky methods, it gives as perfect preparations as absolute alcohol in one-half hour and brings out the neutrophile granulations in better outline. A longer fixation than three to five minutes does no harm; and a shorter one, especially if the fixative is the solvent for the stain, will give fair results, the outline of the cells being sharper the longer the fixative is allowed to act.

(4) Formalin.

This fixative may be used as a 1 per cent. solution in 95 per cent. alcohol and allowed to act for one minute, as Benario advocates, but the writer has had much better success with the 0.25 per cent. solution in 95 per cent. alco-

hol for one minute, as Fitcher and Lazear suggest. Allow the fixative to act for one minute, wash in water, and dry between filter-paper. Some writers advocate the direct treatment of the fixed smear with the stain without an intermediate washing and drying, but I have never found the results as good by this method. Instead of the formalin solutions, the vapors may be used by placing the specimen under a bell jar with a few drops of 40 per cent. formalin and allowing the fixative to act for one to five minutes.¹ The normal staining of the cells is not always as clear as could be desired after the use of such a fixative.

A large number of inorganic fixatives have been advised, among them being mercuric chlorid, chromic acid and osmic acid, but these fixatives are much more apt to produce artefacts than are the others mentioned above. While these latter fixatives give good demonstrations of the nuclear structures and of mitotic figures, the granules are very imperfect; but chromic acid has many advantages as a fixative in the study of the chromatin elements.

(4) Staining Methods.

(A) General Considerations.

Since the work of Witt, we have recognized that the color of an organic substance is due to the presence of two definite atomic complexes in the molecule of the colored substance. The first of these, the chromophore group, is responsible for the chromogenic properties of the substance, while its influence as a dye is increased by the presence of the second or auxochromic group. The color of the compound is the more intense the more of these groups are present. Groups or atoms which intensify the color of the substance are called, by Schütze, bathochromes, while those which reduce the color are called hypsochromes.

While the dyes, the so-called anilin dyes, used in hematological work are all aromatic derivatives, it must not be assumed that such are alone characterized by staining qualities, as many simple aliphatic derivatives show a definite color and exert some staining property, depending on the presence of the two groups above mentioned. The chief chromophore groups are the CO (carbonyl) group, the CS group, CN, HCN, the $-N=N-$ (azo group),

the $\begin{array}{c} \text{—N—} \\ | \\ \text{—N—} \end{array} \text{O}$ (azoxy group), NO (nitroso group), $\begin{array}{c} \text{O} \\ \diagup \quad \diagdown \\ \text{—N—} \end{array}$ (nitro group),

and the $-N=SO$ group. The most important auxochrome groups are the NH_2 and the OH groups, the former being a much more powerful one than the latter. These auxochrome or haptophore groups make possible the union of the stain with the tissue as a direct salt formation. Substances, which possess a chromophore group and are colored or intensified by the presence of the auxochrome group, are called chromogens. It is, therefore, evident that the effect of an auxochrome depends not only upon its own nature, but also upon that of the chromogen. Through the introduction of H by means of hydrocarbon radicals, new auxochromes are produced and the

¹ See van Herwerden, *Nederl. Tijdschr. v. Geneesk.*, 1919, II, 170; *Jour. Exp. Med.*, 1920, XXXII, 135.

color becomes intensified, the effect being so much greater the higher the molecular weight of the substituting hydrocarbon radical.

As a large number of colored substances have a tendency to form tautomeric compounds, the salt formed by combination with the auxochrome groups may have a different constitution from that of the free base or acid. Many chromophore groups are also capable of forming salts, but only with strong acids or bases. In this formation of salts, by union with the auxochrome or chromophore groups, we must remember that halochromia may be seen. By this is meant that uncolored or weakly colored substances may combine with acids to form salts without the color being due to the presence of a chromophore group. It is essential that the chromophore group possess a double bond of union, as oscillation in one portion of the molecule is thus possible. As the chromophoric as well as the auxochromic radicals may have acid or basic tendencies, it is manifest that the reaction of the substitution product will depend upon the interrelations of the acid and the basic radicals. The best dyes are, therefore, obtained by substitution in one direction, that is, by accumulating either basic or acid groups separately.

It is thus seen that we have two general classes of dyes, the acid and basic, depending upon the preponderance of the total acid or basic groups in the molecule of the dye. It has been shown by Ehrlich that these acid and basic dyes, may be so combined that a third one results, showing neither acid nor basic properties. This class of dyes, known as neutral dyes, is of the greatest importance in hematological work. In them we have not only the staining properties due to the original chromophore and auxochrome groups of both the acid and basic dyes, but also those due to the union of the component groups in the neutral dye. Such dyes are hence called polychrome dyes and are usually soluble in an excess of one of the component mother dyes, generally the acid one. It must be understood that in speaking of a dye as acid, basic, or neutral, we do not refer so much in our staining work to the chemical reaction of the dye, but to the portion of the dye to which the staining is due—that is, to the specific auxochrome and chromophore groups.

Among the basic stains we find methyl green, methylene blue, amethyst violet, neutral red, dahlia, pyronin, thionin, fuchsin, methyl violet, Bismarck brown, alum hematoxylin, and safranin. Most of these stains, depending on the strength of their component groups, color the nuclear substance of both red and white cells, as also the cytoplasm and certain granules of abnormal red and of some normal white cells, the staining being influenced, as Matthews has shown, by the alkaline reaction of the tissues. The most important acid stains are eosin, acid fuchsin, orange-G, indulin, nigrosin, aurantia, and salts of picric acid. These dyes color the red cells and the eosinophile (oxyphile) granulations of the leucocytes. The neutral portion of the dyes color the so-called neutrophile granules of the leucocytes.

In the process of staining, it is a question whether we have to do with purely chemical processes as Knecht's theory assumes or whether the solid-solution theory of Witt or the mechanical theory of surface attraction are accountable for the phenomena observed. It is probable that the salts of the

dyes are decomposed by the cells and that new compounds result from the union of the acid and basic stains and the various chemical entities of the cells. Yet we have instances in which the dye is simply stored in the cell without any chemical union taking place. We must, however, account for the elective character of certain stains by a purely chemical activity, as, for instance, chromatin, which undoubtedly consists of nucleinic acid, always takes a basic stain, even though a neutral compound is used as the staining agent. It is to be remarked that a neutral stain does not color all of the acidophile or basophile substances of the cells of the same tint. Thus, eosinophile granules are differently colored from the oxyphile hemoglobin of the red cells. The neutral portion of the stain has nothing to do with the staining process beyond the coloring of the neutrophile substances in the protoplasm of certain leucocytes.

In the selection of a stain for general hematological work, it is necessary to use a compound stain or, at least, two simple stains, one after the other. A single simple stain colors only a few of the elements and affords no general idea of the blood picture. Indeed, to obtain definite conceptions of the finer structure of the blood, it is necessary to study several slides stained by different methods. It is usual to select for routine work a stain which will reveal the greatest amount of information regarding the histological characteristics of the blood. This is the so-called panoptic staining and is to-day generally accomplished by the use of the various forms of neutral stains. In making the stains for one's self or in buying them in the market, one must be certain that only chemically pure pigments are used and that the solution is made according to the formula with the purest solvent obtainable. It will need only one experience with a poorly made stain to convince the worker of the importance of this detail.

(B) **Methods of Staining.**

In his work the author uses, for routine purposes, the Wright's stain, the eosin-methylene blue, and eosin-hematoxylin stains. These stains have the advantage of giving clear-cut pictures of practically all of the important blood elements along with simplicity of technic. As it is not always possible to secure or even to make a Wright or an Ehrlich triple stain which will give reliable results under all circumstances, the writer feels that the general worker would better use the eosin-methylene blue stain for his daily work. The many modifications of the Romanowsky stain have their advocates, and it must be said that they yield reliable results when they are working properly, but no one can say when they will go wrong or how to make them work right when once they do give poor results.

Eosin-Methylene Blue.

A number of methods of using these two simple stains have been advocated by such workers as Chenzinsky, Ehrlich and Lazarus, von Willebrand, Plehn, Aldehoff, and Gabritschewsky, but the writer has found the method advocated by Müllern¹ to be the most generally applicable and, if properly applied, the most reliable modification. By it we are able to stain all of the

¹ Grundriss der klinischen Blutuntersuchung. Wien, 1909.

blood elements, including the neutrophile granules and obtain, thus, a panoptic picture whose findings are not excelled by those of the various modifications of the Romanowsky stain.

Technic.

(a) Fixation of the smear in pure methyl alcohol for three minutes. (b) Preliminary staining in $\frac{1}{2}$ per cent. alcoholic (70 per cent.) solution of Grüber's "french pure" eosin from three to five minutes. (c) Wash in distilled water and dry between filter-paper. (d) Lay the slide in a carefully measured and well-mixed solution of 20 drops of $\frac{1}{4}$ per cent. aqueous solution of methylene blue (B. pat.) and ten drops of the above eosin solution for one-half to one minute. (e) Wash quickly and briefly with distilled water and dry at once between filter-paper or over the flame. (f) Mount if desired in Canada balsam or examine directly with a high-power lens.

This stain shows the red cells and the eosinophile granules of the leucocytes of a bright red tone, the neutrophile granules pink to bright red (distinguished from the eosinophiles by their smaller size), the nuclei, mast-cell granules, bodies of the lymphocytes, platelets, malarial organisms, trypanosomes, and filaria varying shades of blue. The preliminary staining with eosin serves the purpose of bringing out the neutrophile granules more clearly while the basophile granulations are of course unaffected by such treatment. It is probable that the later staining with the eosin-methylene blue mixture has the same characteristics as the neutral stains to be mentioned later. While this stain has the advantage of simplicity, reliability, and panoptic power of staining, it is somewhat inferior to other stains in bringing out some forms of the malarial organism, owing to its lack of chromatin staining qualities. The writer has found that the eosin and methylene blue above mentioned are the best to use in this process and that Türk's advice, regarding the use of fairly fresh eosin solutions and of old methylene-blue solutions, is warranted. The blood preparations should not be over a few days old to show the best pictures, as those over a week old may show a diffuse plasma staining and the neutrophile granules not clearly differentiated. If the nuclei do not stain well with the methylene blue as many writers claim, make a second preparation fixing somewhat longer in methyl alcohol. By following out these precautions the worker will be rewarded with beautiful specimens. This method is to be recommended for all routine examinations.

Eosin Hematoxylin.

This stain is especially important in cases in which the nuclear structures are to be studied.¹ It stains the nuclei beautifully, showing their finer structure, karyokinetic figures, and pycnotic qualities, as well as the basophile granules of both red and white cells. The solutions required are: (1) $\frac{1}{2}$ per cent. Grüber's blood eosin in 70 per cent. alcohol. We may use, with equally good results, the eosin mentioned in the previous method. (2) Delafield's hematoxylin, the formula of which is

¹ See Williams (Jour. Am. Med. Assn., 1913, LXI, 1627), who describes a modification of this stain which he calls "invariable;" also Grant and Wilson, Jour. Lab. and Clin. Med., 1921, VI, 593.

Hematoxylin crystals,	4 grams.
Alcohol (absolute),	25 c.c.
Ammonium-alum crystals, C. P.,	52 grams.
Distilled water,	400 c.c.
Glycerin, C. P.,	100 c.c.
Methyl alcohol, C. P.,	100 c.c.

Rub up the hematoxylin crystals with the alcohol until they are dissolved and place the solution in a loosely corked glass bottle, allowing it to stand exposed to the light for four days. Dissolve the ammonium-alum in the water and allow it to stand exposed in the same way for four days. At the end of this time mix the two solutions, shake thoroughly, and filter at the end of three hours. Add the glycerin and methyl alcohol to the filtrate and allow this to stand overnight. Filter the mixture, place it in a clear bottle, and allow it to ripen, exposed to the light for six weeks, when it is ready for use.

Technic.

Stain the specimen with the eosin solution for one-half minute, and wash in water. Without drying place the slide in the hematoxylin solution for one to three minutes, the time varying with the particular stain and with the experience of the worker. Wash with water, dry, and mount. This stain does not give as good results as does the former method, but is to be especially recommended when the nuclear structures are to be studied.

Ehrlich's Triple Stain.

In the literature of hematology we find the expressions "triacid" and "triple stain" used synonymously. The triacid stain, as originated by Ehrlich, was a mixture of equal parts of saturated solutions of indulin, nigrosin, and aurantia, and was used to differentiate the eosinophile granules. When used synonymously in these days, the triple stain is always meant. The composition of this latter stain is as follows, made up of two acid and one basic stain:

Saturated watery solution of orange-G,	13-14 c.c.
Saturated watery solution of acid fuschin,	6-7 c.c.
Distilled water,	15 c.c.
Alcohol (absolute),	15 c.c.
Saturated watery solution of methyl green oo,	12.5 c.c.
Alcohol (absolute),	10 c.c.
Glycerin,	10 c.c.

The pigments must be chemically pure and the solutions must be added in the order named, the methyl-green solution being added drop by drop with constant stirring.

This stain is difficultly prepared, being usually a failure. The writer would, therefore, advise the worker to obtain it already made; and even then it may not prove satisfactory. The correct stain should have a russet-brown color and should not show any trace of a precipitate. It improves up to a certain point with age but, after a time, precipitates invariably occur, making it useless. It should never be filtered or shaken, the solution to be used being taken from the center of the bottle with a dropping pipet.

Technic.

The smear should be fixed by heat as previously described. After proper fixation, cover the smear with the stain and allow it to act for one to ten minutes as the experience of the worker with the particular stain may indicate. Wash with distilled water, dry, and mount. One advantage of this stain is that it cannot overstain, those films appearing so being underheated, while those understained are overheated.

The stain shows the red cells of a buff or orange color, without the slightest shade of red (a reddish tint is given with underfixed specimens while a yellow tone is shown by those overheated), the nuclei of the leucocytes a dark green, those of the normoblasts black, the neutrophile granules a lilac color (though some occasionally show a reddish tinge), and the eosinophile granules of a crimson tone.

This stain was introduced as specific for the neutrophile granules, but its disadvantages are too numerous to warrant its recommendation as a routine stain. It is a poor nuclear stain, does not show the structure of the normal mononuclear leucocytes, does not stain the basophile granules, nor the malarial or other parasites. For a reliable preparation, showing those features for which it is especially adapted, a proper fixation is an absolute essential.

On account of the lack of nuclear staining with the triple stain, Pappenheim has substituted methylene blue or methylene azure for the methyl green, and eosin for the acid fuchsin. The writer has had no experience with this modification and cannot, therefore, speak regarding its value.

Polychrome Methylene-blue-eosin Stains.

These stains are very numerous, each having its advocates. They are easy to use, contain a reliable fixative, and give satisfactory results, but are not always obtainable or easily prepared. They are the stains which give, perhaps, the best panoptic results and are especially serviceable in the study of the malarial organism and other parasites as many of them contain chromatin-staining elements. The granulations of the leucocytes are not as well marked in all cases as they should be, so that for a complete study of the various types of granules in the cells several stains would better be used.

Romanowsky had found that the addition of a watery eosin solution to an aqueous methylene-blue solution, until an insoluble precipitate began to form, gave rise to new staining properties of the solutions, in the sense that the chromatin substance of malarial organisms was stained a beautiful red. The specific staining properties of this mixture were later found to be due not to a combination of eosin and methylene blue, *per se*, but rather to the formation of a new compound between eosin and an impurity or decomposition product in the methylene blue, namely, methylene azure. Jenner in his stain makes use of a methyl-alcohol solution of the isolated precipitate, the eosinate of methylene blue, which lacks the red chromatin staining element. The same may be said of the May-Grünwald stain. •

In making up these polychrome stains it is not general to use the pure methylene azure and eosin, but rather solutions of methylene blue containing a variable amount of the methylene azure to which eosin is added. Recently

Wilson,¹ in a careful study of the methylene-blue-eosin combinations, has shown that very little methylene azure and methylene violet exist in the stains as commonly employed. He finds evidences of at least four staining bodies in such mixtures, namely, the eosinate of methylene blue, eosinate of methylene violet, eosinate of methylene azure, and eosinate of thionin.

Wright's Stain.

This stain is a much improved modification of the Leishman stain,² as its method of preparation requires but a few hours and as the resulting dye is somewhat more panoptic than is that made by the Leishman method.

Preparation.

To a 0.5 per cent. aqueous solution of sodium bicarbonate add methylene blue (B. X. or "medicinally pure") in the proportion of 1 gm. of the dye to each 100 c.c. of the solution. Heat the mixture in a steam sterilizer at 100°C. for one full hour, counting the time after the sterilizer has become thoroughly heated. The mixture is to be contained in a flask, or flasks, of such a size and shape that it forms a layer not more than 6 cm. deep. After heating, allow the mixture to cool, placing the flask in cold water if desired, and then filter it to remove the precipitate which has formed in it. It should, when cold, have a deep purple-red color when viewed in a thin layer by transmitted yellowish artificial light. It does not show this color while it is warm. To each 100 c.c. of the filtered mixture add 500 c.c. of a 0.1 per cent. aqueous solution of "yellowish, water-soluble" eosin and mix thoroughly. Collect the abundant precipitate, which immediately appears, on a filter. When the precipitate is dry, dissolve it in methyl alcohol (Merck's "reagent") in the proportion of 0.1 gm. to 60 c.c. of the alcohol. In order to facilitate solution, the precipitate is to be rubbed up with the alcohol in a porcelain dish or mortar with a spatula or pestle. This alcoholic solution of the precipitate is the staining fluid. It should be kept in a well-stoppered bottle because of the volatility of the alcohol.³

Technic.

Cover the film with a noted quantity of the staining fluid by means of a

¹ Jour. Exper. Med., 1907, IX, 645.

² *Leishman Stain.* Prepare a 1 per cent. aqueous solution of medicinal methylene blue (Grübler) and add 0.5 per cent. sodium carbonate; heat for 12 hours at 65°C. and keep for 10 days at room temperature. Prepare a 0.1 per cent. aqueous solution of "yellowish, water-soluble" eosin. Mix equal volumes of the two solutions and leave for 10 to 20 hours, shaking the mixture at frequent intervals. Filter, wash the precipitate with distilled water until the washings are a pale blue, dry on the filter and powder the dried residue. The staining solution is prepared by dissolving 0.2 gram of this powder in 10 c.c. of methyl alcohol. This stain is used precisely as the Wright stain, the blood-picture being very similar.

³ Jour. Am. Med. Assn., 1920, LV, 1970. Cunningham (Arch. Int. Med., 1920, XXVI, 405) combines a vital stain with the Wright stain for the purpose of staining reticulated red cells. A small drop of a 0.3 or 0.5 per cent. aqueous or alcoholic solution of brilliant cresyl blue is placed on the end of a clean slide and smeared around over an area 1.5 cm. in diameter, and permitted to dry. A drop of fresh blood is placed on a clean cover-slip and this dropped face down on the stained area of the slide, when the blood quickly spreads to the edges. The cover glass and the slide are then separated as in making blood smears and are permitted to dry, when the blood turns a dirty greenish blue color. The slide or cover glass is then stained with Wright's stain in the usual manner, too vigorous washing of the preparation being avoided, as this may cause the reticulum to lose some of its stain. The reticulum is stained a deep or light blue and gives a striking picture in its contrast with the pink protoplasm of the cells.

medicine dropper. After one minute add to the staining fluid on the film the same quantity of distilled water by means of the medicine dropper and allow the mixture to remain for two or three minutes, according to the intensity of the staining desired. A longer period of staining may produce a precipitate. Eosinophilic granules are best brought out by a short period of staining. The quantity of the diluted fluid on the preparation should not be so large that some of it runs off. Wash the preparation in water for thirty seconds or until the thinner portions of the film become yellow or pink in color. The experience of the worker may be such as to demand longer decolorization with any particular specimen. When the desired degree of differentiation is reached, dry the specimen quickly between filter-paper, mount if desired, and examine first with the low-power lens to observe the staining effects, and then with the high-power lens for the more minute study. When searching for malarial parasites, the decolorization would better be of short duration, as the chromatin suffers to a great extent in this process.

This stain shows the red cells colored either orange or pink (depending on the time of decolorization), nuclei of leucocytes blue or dark lilac, neutrophile granules lilac, eosinophile granules red or pink, fine basophile granules deep blue, large mast-cell granules purple, protoplasm of the lymphocytes robin's-egg blue, blood-plates deep blue or purple, bacteria blue, malarial and other parasites blue, the chromatin element varying from lilac to ruby-red to black. Polychromatophilia and granular degenerations are well shown, the granules being blue. This stain is very useful in studying lymphocytes, mast cells, blood-plaques, and the finer structure of the malarial organism; but the leucocytic granules, at times, are not sufficiently differentiated.

Giemsa Stain.

Giemsa has shown that the complicated methods of preparing the dyes may be dispensed with by the use of the pure staining substance extracted from the polychrome methylene blue, namely, the methylene azure. If this pure pigment be used the stain becomes a pure chromatin one. It is advisable, therefore, to use Grüber's azure II, a combination of equal parts of methylene azure and medicinal methylene blue. The formula of the Giemsa-stain is as follows:

Azur II-Eosin,	3.0 grams.
Azur II,	0.8 gram.
Glycerin (C. P.),	125.0 grams.
Methyl alcohol (C. P.),	375.0 grams.

Grind up the dyes in the alcohol and then add the glycerin.

Technic.

Fix the films in methyl alcohol, and stain for fifteen minutes in a mixture of 15 drops of the stain to 10 c.c. of distilled water. If necessary, a trace of sodium carbonate may be added to the water to intensify the basic stains. Wash in water, dry, and mount. It is to be remembered that oil of cedar will bleach these specimens quite rapidly, so that it is advisable, if the films are to be kept, to stain them upon the slides so that mounting becomes unneces-

sary. Strong light should not be allowed to act on these stained films for any length of time as the chromogen stain fades rather quickly under these conditions. The various elements are stained as with the Wright stain, but the neutrophile granules are often not well defined.¹

Pappenheim's Stain.

Pappenheim (*Technik der klin. Blutuntersuchungen*, Berlin, 1911; *Folia Hæmat.*, 1912, XIII, 339) advocates as the best and most serviceable stain the combination of the Giemsa stain as follows: Fix the blood film by covering it with May-Grünwald stain (Wright's answers as well) for 3 minutes, add to this stain on the slide an equal amount of distilled water and allow it to act for 1 minute. Pour off the fluid but do not wash. Now cover the stained film with dilute Giemsa solution (15 drops of stain to 10 c.c. of distilled water) and allow to act for 15 minutes. Wash thoroughly and dry, but not over the flame. Mount, if desired, in neutral Canada balsam. The result is extremely pan-optic. The nuclei stain violet. The lymphocytes, monocytes and undifferentiated lymphoidocytes have a delicate blue cytoplasm. The mast-cell granulations are ultramarine blue. Eosinophiles have brick-red granulations and the neutrophiles a rose-colored cytoplasm, while their granules are indefinite.

Specific Stains for Malarial Organisms.

While the above stains all give good results with the malarial organisms, enabling one to make a diagnosis from an examination of the smear, yet certain other stains are often of advantage in that little else is stained and thus the confusion arising from indefinite staining is obviated.

Thionin Stain (Futcher and Lazear).

Add to 100 c.c. of 2 per cent. carbolic acid 20 c.c. of a saturated solution of thionin (Lauth's violet) in 50 per cent. alcohol, and allow this mixture to ripen for a few days.² Fix the specimen by the formalin method given above and stain the smear for 10 to 15 seconds. Wash in water, dry, and mount. These preparations do not keep indefinitely, usually fading within a year.

The plasmodia are shown as deep purple, irregular masses enclosed in the faint green red cells. The hyaline forms show as reddish-violet ring-like bodies. The basophile granules and the nuclei are the only other elements showing any particular staining qualities.

Nocht's Stain.

This is essentially a Romanowsky stain and is made as follows: Add two or three drops of 1 per cent. aqueous eosin solution to 2 c.c. of water. To this diluted eosin solution polychrome methylene blue is added, drop by drop, until the red color of the eosin is only faintly visible (the polychrome

¹ See McJunkin (*Jour. A. M. A.*, 1915, LXV, 2164) for a new polychrome stain. Also, see Martini. *Rit. Med.*, 1916, XXXII, 1158; Krauss, *Jour. Lab. and Clin. Med.*, 1916, II, 138; Lago, *Porto Rico Med. Assoc. Bull.*, 1919, XIII, 62; Agulhon and Chavannes, *C. R. soc. biol. Paris*, 1919, LXXXII, 149; McJunkin (*Jour. A. M. A.*, 1920, LXXIV, 17) has devised a benzidin-polychrome stain which permits of great accuracy in the identification of the leucocytes, as the granules of the neutrophils, endothelial leucocytes and eosinophiles stain dark brown. See, also, Polletini, *Policlinico*, 1920, XXVII, 791.

² See Lesieur and Jacquet, *C. R. soc. biol.*, Paris, 1919, LXXXII, 267.

methylene blue is a 1 per cent. solution of methylene blue chemically changed by heating with $\frac{1}{2}$ per cent. sodium carbonate solution for two days at 55°C.).

Technic.

Fix the film in methyl or ethyl alcohol and lay it face downward in the above mixture for five to ten minutes. Wash with water, dry and examine. The picture is the same given by Wright's stain, except that the nuclear transformations and the chromatin substance are better differentiated, being stained a bright red color.

The many other stains for the examination of fixed specimens will be passed over, as the writer finds the above stains applicable to practically all routine work. Many writers prefer different stains from those mentioned, but these stains do not seem to have any advantages which would warrant their use to the exclusion of those given above. Special stains used for bringing out certain granules and deformities will be discussed under later headings.

Vital Staining.

Although the examination of stained specimens of the blood is usually made with the dried and fixed smear, excellent results obtain when the fresh blood is stained without previous fixation. It is true that a "vital" staining of the blood-cells does not actually take place, as the dyes are decolorized by the reducing and oxidizing action of the living cells. However, a "post vital" staining, that is the staining of whole cells or portions of the cells, after their removal from the circulation and before the death of the cell results, may be accomplished in several ways.

We may either add to the fresh drop of blood a few crystals of the stain, as advised by Arnold, and note the staining of certain leucocytic granules and nuclei and reticular structure of many erythrocytes; or we may first dry a staining solution upon the slide, cover this dry stain with a drop of fresh blood, adjust the cover-glass, and seal this to the slide with wax. Some workers prefer to use the hanging-drop slide in the preparation of these fresh specimens.

The stains which may be used for vital staining of the nuclei, granules, and plates are methylene blue, toluidin blue, thionin, neutral violet, Capri blue, Nile blue, brilliant-cresyl blue, Janus green, and paraphenyl blue. Of the protoplasmic stains we have fuchsin, acridin red, pyronin, safranin, and neutral red.

While the results derived from this method of staining are not as numerous as those of the more usual method, yet it affords much valuable information regarding the vital properties of the cells and regarding the normal structure and the circulatory changes of the cells.¹ This method has been used extensively by Ito, Rosin and Bibergeil, Levaditi, Cesaris-Demel, and Pappenheim. Their results lead us to assume that further study will give us much valuable information concerning details of structure both of the cell and of the nucleus. It may be possible by this method to differentiate between certain forms of

¹ See Luzzatto and Ravenna (*Folia Hæmat.*, 1912, XIII, 102) for a discussion of the numerical relations of the granular and non-granular erythrocytes as shown by this method. Also, Schulemann, *Biochem. Ztschr.*, 1917, LXXX, 1.

degeneration of the cell which are now known only indefinitely under the names of metachromatic and polychromatic staining.¹

(5) Erythrocytes.

(A) Appearance and Structure.

In fresh, normal blood the red cells, or erythrocytes, appear as thin, flattened, homogeneous, biconcave, nonnucleated, discoid bodies with a sharply defined regular outline and a clear semitransparent center. In some cases they may appear distinctly cup- or bell-shaped.² The cells show, when examined singly, a pale greenish-yellow color but, when more thickly grouped, exhibit a reddish tint. The degree of color in these cells depends upon their hemoglobin content, the clear central area becoming larger and the entire cell



FIG. 144.—Normal blood showing rouleaux formation and fibrin network. (Da Costa.)

becoming paler as the hemoglobin decreases. The loss of hemoglobin may be so great as to lead to the formation of the so-called "pessary" form, in which only the periphery of the cell is apparent. This central pale area varies much in individual cells and is not at all evident in those which are flattened out. In dry specimens, if thinly spread, the cells are circular, the normal biconcavity is obliterated, and a uniform stain is observed. If the smear be thick the central pale area is observed.

In chlorosis and secondary anemias, the color of the cells is usually uni-

¹ See Ross (Induced Cell-reproduction and Cancer, Philadelphia, 1911, and London, 1912) for the use of agar films containing stains and activating salts (auxetics) in this connection. See, also, Anitschkow, *Med. Klin.*, 1914, X, 465; Barbaro, *Gazz. d. osp.*, 1914, XXXV, 681; von Möllendorff, *Deutsch. med. Wchnschr.*, 1914, XL, 1839; Höber, *Biochem. Ztschr.*, 1914, LXVII, 420; Lindborn, *Nord. Med. Arch.*, 1914, XLVII, 1; Evans and Schulemann, *Science*, 1914, XXXIX, 443; *Folia Hæmat.*, 1915, XIX (I), 207; Rubino, *Rif. Med.*, 1915, XXXI, 533; Traube, *Biochem. Ztschr.*, 1915, LXIX, 309.

² See Jordan, *Proc. Soc. Exper. Biol. and Med.*, 1915, XII, 167; Arey, *Science*, 1916, XLIV, 392; Mas y Magro, *Sig. Méd.*, 1916, LXIII, 466 and 482. Wyss (Schweiz, *med. Wchnschr.*, 1920, L, 266) believes these cells to be round or egg shaped in the vital state and that the escape of oxygen from them when the blood issues from the vessels causes them to collapse into concave forms. See Greenthal and Brown, *Arch. Int. Med.*, 1922, XXX, 99.

formly paler than normally, while in pernicious anemias the color may be even deeper than normal. These are the usual but not invariable pathologic findings. In malarial conditions, discolored cells are often observed in the fresh specimens, the bronzed or "brassy" tone often drawing attention to the presence of a parasite of the estivoautumnal type or of the quartan form.

The red cells show a marked tendency to cohere to one another in more or less regularly arranged piles, forming long rows (*rouleaux*), like rolls of coin piled up face to face. The exact cause of this phenomenon is unknown, but it may be dependent on the presence of the fatty membrane surrounding the cell, as Peskind's findings show that the red cells are enveloped by a layer composed of lecithin, cholesterin, and a nucleoprotein.¹ In certain pathologic conditions this normal *rouleaux* formation is increased and in certain ones is decreased. The diminished *rouleaux* formation is observed in conditions associated with increased viscosity of the cells, as observed in most inflammatory diseases and in the anemias due to malignant disease. This hyper-viscosity of the cells has not at present much clinical significance, but may be shown to be of importance when our knowledge concerning the normal viscosity of the blood becomes more extensive.

The structure of the erythrocytes is still an unsettled point in hematology. Neither membrane nor stroma have been fully demonstrated yet after the hemoglobin has been removed from the cells by hemolysis, a stroma may be definitely seen which could hardly be called an artefact. Schäfer assumes that the hemoglobin is held in firm combination by chemical union with other albuminous constituents of the cell and supported by a stroma similar to that of Peskind's outer membrane. That some sort of an outer membrane does exist would seem to be proven by the experiments on hemolysis which have shown that many substances penetrate the cell producing hemolysis, while others in the same concentration have no such effect. It is hard to believe that a selective vital activity is at work here, as the results follow too closely the laws of physical chemistry as applied to diffusion and osmosis. The corpuscles are very elastic and contractile so that rapid and marked temporary distortions of shape are possible under the influence of variations in the composition of the circulating plasma.

As the blood dries various changes in the appearance of the red cells are observed. These changes, known as crenation, are due to the evaporation of water and depend upon the quantity of air which comes in contact with the specimen as well as upon the length of time this influence acts. "The development of one or more small, bright, highly refractile spots in the body of the cell and a slight indentation of the periphery of the cell are the most conspicuous indications of beginning crenation. As the process goes on, more and more of these hyaline points develop, until finally the whole surface of the corpuscle becomes thickly studded with glistening bead-like spines. As the stroma becomes drier and drier, its typical biconcavity and sharply cut outline are lost, contracting strands of the stroma are seen to extend from point to point among the beaded projections, the periphery of the cell changes to a

¹ See Wiltshire, *Jour. Path. and Bacteriol.*, 1913, XVII, 282; Swift, *Jour. Lab. and Clin. Med.*, 1922, VII, 614.

cogged rim, and finally the cell becomes shrunken and shriveled up into a small, many-starred asterisk. Some of the erythrocytes become fragmented and small bits of their stroma are observed to break off and float through the plasma. Others become progressively paler and paler, as the hemoglobin is dissolved out, until complete decoloration occurs. Still others become distorted into designs of every conceivable shape so that their resemblance to the normal cell becomes more remote" (Da Costa). These changes must not be confused with those occurring as a result of pathological changes. Crenation is often induced more rapidly than in normal blood, in the blood of persons suffering from acute infection and from chronic diseases. True ameboid movement of the red cells is sometimes seen as a result of a high-grade anemia.¹

(B) Size and Shape.

The average diameter of these normal human erythrocytes (normocytes) is $7.5\ \mu$, the normal variations being between 6 and $9\ \mu$ (a micromillimeter, $\frac{1}{1000}$ of a millimeter). The size varies depending on the method of preparing the specimen and also upon the osmotic tension of the plasma. Although dwarf and giant cells may occur to a slight degree at all ages, the normal infant blood shows these variations more markedly (3.3 to $10.3\ \mu$, according to Hayem). According to Hamburger, the cells are slightly larger in the venous than in arterial blood. Gram states that the size of these cells varies with climatic conditions, being greater in those of the northern cooler countries than in those of the southern warmer climates. Price-Jones observes that there is a diurnal variation in the size of these cells but is uncertain as to the cause.² The variations in the size of the red cells is very slight in the different sexes.

Pathologically, variation in the size of these cells is a common and important feature. Generally speaking, variations in the cellular size indicate a severe and chronic anemia, while in the more mild acute forms of anemia such variations are unusual. The average size is said to be increased in jaundice, cholera, lead-poisoning, leukemia, congenital heart disease, and cretinism.

Types of Pathological Erythrocytes.

Variation in the normal size of the red cells is indicated by the term anisocytosis. This term does not include the misshapen red cells which appear in the blood as a result of degeneration or of mechanical injury and to which are given the name of poikilocytes.

Microcytes.

These are cells under the normal size, the variations being between $1\ \mu$ and $6\ \mu$, the usual representatives being about $3.5\ \mu$. It cannot be stated at present whether these cells are mere schistocytes (fragments of larger cells) or are perfect cells of degenerative origin. They occur normally in the blood of embryos and infants, but are rare in that of the adult except in pathological conditions. It is certainly true that these undersized cells may

¹ See Krizenecky, *Ztschr. f. allg. Physiol.*, 1915, XVII, 1.

² *Journ. Path. & Bact.*, 1920, XXIII, 371. See also, Holker, *Biochem. Journ.*, 1921, XV, 226.

arise from purely physical causes, as a result of increased osmotic pressure of the plasma as well as from division of undersized mother cells. This latter phase is made possible by the appearance, especially in pernicious anemia, of nucleated reds of corresponding size.

Pathologically, these smaller cells are observed in all severe anemias. As a rule, they stain deeply and evenly, but in some cases of pernicious anemia these cells are deficient in hemoglobin and show irregular staining, yet many of them may have an increased hemoglobin content. Occasionally, but not invariably, we find these undersized cells in chlorosis, in which the hemoglobin is deficient. At times these cells may show polychromatophilia, but this is not the rule. According to Tallqvist, an increase in the number of these microcytes (microcytosis) is an indication of rapid destruction of blood.

Macrocytes.

These are cells above the normal size, the variations being between 10 and 20 μ . Those cells from 9 to 12 μ are called macrocytes; those between 12 and 16 μ are known as megalocytes; while those above 16 μ are termed giantocytes.

These cells are of regular shape, of even staining qualities, and generally without a well defined central clear area. The larger size of these cells may be partly due to the swelling incident to a lowered osmotic tension of the blood, but more probably this increase in size is traceable to the origin of the macrocytes from the large nucleated reds of the bone-marrow. These cells may show an excess or deficiency in hemoglobin, the former characteristic being observed in the primary pernicious anemias, while the latter is evident in the secondary forms. Morris and Thayer¹ report ameboid movements in these cells.

Pathologically, the presence of these various forms of macrocytes, giving rise to the condition of macrocytosis, indicates a severe and unusually chronic anemia. They are most frequently seen in pernicious anemia, in which the largest cells are sometimes the darkest and some of the microcytes are exceedingly pale. These large cells do occur, however, in leukemia, cholemia, and chlorosis, being frequently pale or "chlorotic" and "dropsical." These "dropsical" cells are not sufficiently numerous, however, to change the volume index, as Capps has shown, of a secondary anemia to one shown in true pernicious anemia.

Poikilocytes.

These are misshapen red cells of large or of small size, the varieties of such deformities being numerous. The presence of poikilocytes in the blood is known as poikilocytosis (first described by Damon) and is closely related to crenation as in both cases the cells may be similarly misshapen. The former is a pathologic process demonstrable the moment the blood is taken, while the latter is a physiological process appearing only after the blood has been in contact with air for some time.

Poikilocytes arise in several ways. First, faulty technic, especially pressure on the cover-glass of the fresh specimen, will give rise to fragmenta-

¹ Arch. Int. Med., 1911, VIII, 581.

tion of some of the corpuscles into small spherical masses, dagger-shaped bodies, and small elongated rods (pseudo bacilli of Hayem). Such fragmentation is indicative of lowered vitality and feeble powers of resistance of the cells. If the cover-glass be moved after the cells have spread, a large number of them will be distorted into oval or pear-shaped forms, the long axes of which usually point in the same direction. Secondly, true poikilocytes, or cells misshapen while in the circulation, are probably due to amoeboid motion of a portion or the whole of a cell or to alterations in the plasma. These misshapen cells are usually pear-shaped with a budded projection at one or more poles. Cells may be seen which resemble a tennis-racket, a kidney, tomahawk-blade, dumb-bell, or anvil, while oval forms are especially observed in pernicious anemia and are considered by Cabot of diagnostic importance. Poikilocytosis is an indication of severe anemia with degenerative changes in the red cells; although it is not characteristic of any single disease, it is found more frequently in pernicious anemia and leukemia.

(C) Nucleation.

Nucleated red cells may be considered pathological at any period of extrauterine life, although they are usually found in the blood of the child during the first few days of life. These nucleated reds are always found in the bone-marrow, the normal and large forms being quite distinctive. The large form is the oldest and gives rise by cell division to the smaller cell or the normoblast. It is probably true that the nonnucleated red cells are derived from the nucleated form, but the denucleation takes place before the normal cells reach the blood. Just how we are to explain the disappearance of all trace of the nucleus from the normal erythrocyte is a question, but the general consensus of opinion seems to be that the nuclear material gradually fades within the cell, although some slight evidence of extrusion of the nucleus can be advanced: Although our ordinary methods show no such evidence, King¹ has recently introduced a method by which he demonstrates stainable remnants of a nucleus in practically every red cell in the normal adult blood.

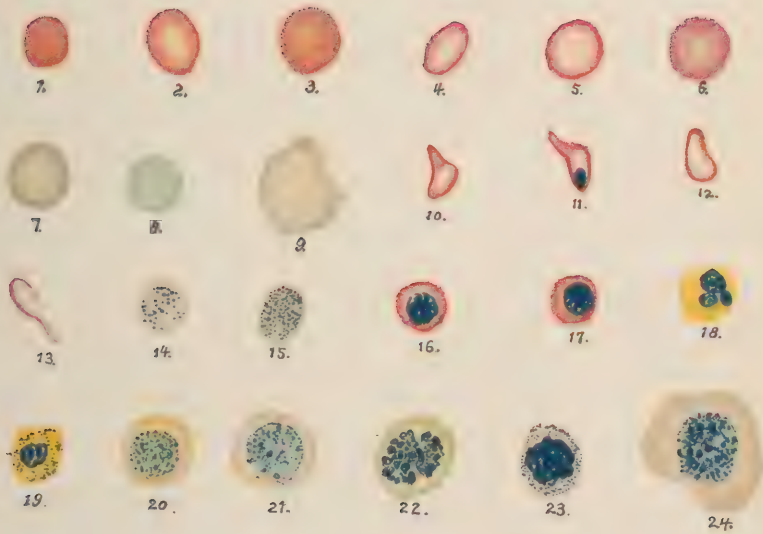
Normoblasts (Trachyochromatic Erythroblasts).

These are nucleated red cells similar in size, shape, and color to the normocytes. They do not usually show a biconcave form and do not unite in rouleaux. The protoplasm of the cell is usually regular in outline, stains more intensely than does that of the normocyte and frequently shows evidence of polychromatophilia, although it is normally orthochromatic.² In myeloid leukemia, cells are frequently observed in which the protoplasm presents a ragged outline and may even, in some cases, be so degenerated as to show only a small fragment attached to the nucleus. These latter cells are practically always polychromatophilic. This type of small cell might be called a

¹ Jour. Med. Research, 1911, XXIV, 91.

² Certain small bodies (Mitochondria) of a lipoid nature are regularly present in nucleated red cells and in non-nucleated reds of the bone marrow as well as in non-nucleated forms in circulating blood of man in some diseases in which the blood forming organs are stimulated to increased activity. These bodies may be stained with Janus Green and other stains. See Cowdry, Internat. Monatschr. f. Anat. u. Physiol., 1914, XXXI, 267; Am. Jour. Anat., 1916, XIX, 423; Shipley, Pol. Hæm., 1916, XX, 61; Sappington, Arch. Int. Med., 1918, XXI, 695; Cowdry, Carnegie Inst., Pub. 271, 1918, p. 39; Key, Arch. Int. Med., 1921, XXVIII, 511.

PLATE XVIII.



Katharine Hill

TYPES OF RED CELLS.
(WRIGHT'S STAIN. ZEISS OCULAR 4, OIL IMMERSION OBJECTIVE.)

- 1—NORMAL CELLS.
- 2-3-4—NORMAL CELLS AS SEEN WITH DIFFERENT FOCUS.
- 5—SO-CALLED "PESSARY FORM."
- 6-7-8—POLYCHROMATOPHILIC CELLS.
- 9—MACROCYTE.
- 10-12-13—POIKILOCYTES.
- 11—POIKILOBLAST.
- 14-15—PUNCTATE BASOPHILIA IN RED CELLS.
- 16-17—NORMOBLASTS.
- 18—NORMOBLAST WITH PYKNOTIC NUCLEUS.
- 19—PUNCTATED NORMOBLAST.
- 20-23—MEGALOBLASTS.
- 24—GIGANTOBLAST.

microblast, which is the rarest form of erythroblast and corresponds in size to the microcyte. Moreover, we may find such cells or fragments of cells attached to a nucleus, such cells corresponding in size and shape to the poikilocytes and being termed poikiloblasts.

The nucleus of the mature normoblast (Howell's mature nucleated red) has a diameter of about one-third that of the cell, is densely stained, homogeneous, sharply defined, spheroidal in shape, and without any decided chromatin network (the so-called pycnotic nucleus). It is situated rather toward the periphery than in the central portion of the cell and is surrounded by a clear zone shading off into the cellular protoplasm. Occasionally the nucleus is observed resting upon a margin of the red cell or even extruded entirely from it probably as a result of degeneration of the surrounding protoplasm (Pappenheim). These nuclei often show amitotic figures, being subdivided into two or more lobes or fragments which may be connected by strands of chromatin. These mature forms may show all gradations in which the chromatin network becomes more and more evident, until we reach the very immature forms (Howell's immature nucleated reds). These latter cells are somewhat larger than the mature forms and are somewhat lighter in color; the nucleus is relatively larger and is composed of delicate faintly basic chromatin fibers radially arranged, and frequently showing mitotic figures.

These two types of cell are the forerunners of the normocyte and their appearance in the adult blood is indicative of increased activity of the hematopoietic organs, especially of the bone-marrow, either as a result of a poor condition of the blood itself or as a direct disease of the blood-forming organ.¹ These cells are most commonly seen in the milder forms of anemia, chlorosis, and acute anemia from hemorrhage, inanition, or organic disease. In the severe types of anemia they are constantly met with and are usually associated with the larger megaloblasts. During the course of severe anemia, especially in chlorosis, there obtains a periodic increase of normoblasts and of leucocytes lasting several days. This is followed by a marked increase in the normal number of red cells, giving rise to the condition described by von Noorden as a "blood crisis." The normoblasts disappear, the blood count falls off, and a second crisis may obtain. This condition is considered as a transitory attempt on the part of the bone-marrow to regenerate the blood. Such a crisis is not always a sign of improvement, but it does demonstrate that the type of blood formation is becoming physiological and that recovery may follow.

Megaloblasts (Amblyochromatic Erythroblasts).

These are cells of larger than normal dimensions, corresponding in size to the macrocytes and varying from 9 to 20 microns in diameter, if exceeding 20 microns they are known as the gigantoblasts of Ehrlich. Occasionally some cells are seen which are no larger than a normoblast, but in which the nucleus shows definite characteristics which should enable one to classify the cell as a megaloblast (Pappenheim).

¹ See Weller, Jour. Med. Res., 1915, XXXIII, 271; Gilbert, Arch. Int. Med., 1917, XIX, 140; Drinker, Drinker and Kreutzmann, Jour. Exper. Med., 1918, XXVII, 249, and 383.

The protoplasm of the megaloblast often appears swollen and enlarged (dropsical). The cell is usually circular or oval, but it is easily deformed, giving rise to irregular-shaped bodies. Although it usually contains an excess of hemoglobin, it may show a deficiency. It is usually polychromatophilic, the shade of cellular staining varying from yellow to purple. These various color tones may not be regular, but may be varied by tints of almost any shade in the same cell.

The nucleus of the megaloblast is very large, varying between 6 and 10 microns. This may be situated either centrally or somewhat peripherally. It shows a great variety of forms appearing as a vesicular body with intranuclear network and nodal thickening, but without nucleoli. It may rarely be pycnotic, may show mitoses or many stages or karyorrhexis with fragmentation, vacuolation, fading of the segments of the nuclei, as well as minute subdivisions into fine basic staining particles widely scattered in the cell (Ewing). It is frequently poorly defined and shows feeble basic staining qualities. It may be sharply differentiated from the body of the cell by a distinct white margin which is thrown into relief by the deeper staining of the nuclear and cell-substances. Occasionally the nucleus is overlooked owing to the polychromatophilic properties of the cell, which do not always permit of clear differentiation of nucleus and cell substance. Careful examination of the nuclear structure with its wide-open meshwork, should, however, prevent the mistake of classifying this cell as a large lymphocyte.

The clinical significance of the megaloblast is more or less in doubt. As such cells are foreign to the blood of an adult and as they are not present beyond 15 per cent. of the total nucleated reds in the marrow, according to Emerson, it must be assumed that their presence in considerable number is indicative of a reversion to an embryonal type of blood formation, or at least denotes an arrested development of normal cells and in consequence an increased production of these abnormal types. Megaloblasts are, therefore, evidences of degeneration of blood-forming organs, while normoblasts are significant of regeneration of blood. A few of these cells, along with a larger number of normoblasts, has no special significance in cases of severe anemia, being found in small numbers in any variety of anemia. If, however, the majority of the nucleated red cells be megaloblasts, especially if giantoblasts be present and unequal mitoses be observed, a diagnosis of primary pernicious anemia seems justified. It may be considered that the number of erythroblasts has no special significance as regards the severity of a particular case, but that it indicates merely the effort on the part of the bone-marrow to overcome the effects of blood destruction. The appearance of megaloblasts may, therefore, be regarded as an evidence of incomplete formation of the younger elements and, not necessarily, as an unfavorable sign. In the anemia following infection with *bothriocephalus latus*, the specific toxins produce a megaloblastic degeneration of the bone-marrow, so that the blood picture may assume the characteristics of a severe pernicious anemia. Ehrlich and Lindenthal have reported a case of nitrobenzol poisoning, in the later stages of which the

megaloblasts out-numbered the erythroblasts. Thayer¹ is the first to report the finding of ameboid movements in a megaloblast in a case of Addisonian anemia.

(D) Number of Red Cells.

The normal number of red cells is generally regarded as 5,000,000 per cmm. in the blood of the adult male, while the normal value for the female is 4,500,000. These figures are purely arbitrary, but they serve as an approximate basis upon which one may form his opinion as to the probable normality of a specific specimen of blood.² We frequently observe marked variations in the red count under the influence of both physiologic and pathologic conditions. These variations may be due to an actual increase or decrease in the number of cells or to a change in the volume of the plasma as discussed under Total Volume of Blood on page 429. It is not uncommon to find much higher counts than these normal ones on healthy individuals, especially in those living the "simple life," so that any special count must be a law unto itself and must be considered normal or abnormal only after considering all the factors which may influence the number of cells.

Physiologic Variations.

(1) Sex.

The variations due to sex are not so marked as those from other causes. While the adult woman almost invariably shows a lower blood count than does the adult man, yet we occasionally find the girl showing, before puberty, a somewhat higher count than the boy of corresponding age, and also the woman, after the menopause, showing somewhat higher values than does her brother of similar years. It can hardly be doubted that menstruation, as well as pregnancy and lactation, have some influence in lowering a count in woman at certain periods of life, yet these conditions are simply transient and would not seem to have as much bearing on the question as does the somewhat more hydremic state of the normal plasma of woman.

(2) Age.

The number of the red cells varies more or less with the age of the subject examined. The highest values are usually observed at birth, when the count may run as high as 7,500,000, as in a case observed by the writer, the hemoglobin being practically always over 100 per cent. at this time; but, as a rule, a lower count is noted, averaging about 6,000,000. These high figures are due, to some extent, to the concentration of the plasma at birth through the loss of body fluid before a compensatory intake. The count gradually falls during the first few days and becomes fairly constant about the tenth day, when it is rare to find nucleated red cells. The number of cells is then stated to be somewhat reduced until the age of puberty, when a gradual increase occurs until about 40 years of age, after which a slight decrease may be observed in man. These variations, observed at different ages, are to be regarded as slight and influenced, to some extent, by the many modifications of the plasma as the result of growth and development as well as to the gradual decrease of functional activity after the middle periods of life.

¹ Arch. Int. Med., 1911, VII, 223; Ibid., 1911, VIII, 581.

² See Bing, Ugesk. f. Laeger, 1910, LXXXI, 1483; Bogendörfer and Nonnenbruch, Deutsch. Arch. f. klin. Med., 1920, CXXXIII, 389.

(3) Altitude.

An increase in the number of cells has been observed under the influence of a higher altitude. The count has been shown to increase at the rate of approximately 50,000 cells per 1,000 feet of ascent and to diminish, within 36 hours, at a corresponding rate, this increase or decrease being more marked the more sudden the ascent or descent. Just what factors are at work in causing these changes is undecided.¹ The rise is too rapid to be entirely accounted for by new formation of blood-cells under the influence of diminished oxygen tension, and the fall is not accompanied by signs of destruction of the reds. Weinzirl considers the increased count of high altitudes due to the lowered temperature at these elevations. This factor would seem to have some influence, as counts are frequently observed in which a variation is noted at places showing the same elevation. Change of residence from warm to a cold or from cold to a warm climate may lead to an increase or decrease,² as the case may be, in the number of cells varying from 500,000 to 2,500,000.

(4) Nutrition.

The general nutritive condition of the subject has more or less influence upon the number of red cells per cmm. This statement must not be interpreted to mean that the obese person shows a higher blood count than does his sparer brother. It is well known that obesity is an indication of poor assimilation of food; hence we should expect to find, as we really do, that the muscular person has a somewhat higher count than the obese subject and well-developed robust individuals a larger value than do the poorly nourished patients. Diet has, of course, much to do with the general nutrition, so we find the meat eaters averaging somewhat higher in their percentage of red cells than do vegetarians. Immediately following a hearty meal we may observe a temporary decrease in the number of red cells, but this soon returns to normal, owing to the rapid adjustment of the water content of the blood.³

(5) Exercise.

Active muscular exercise produces a transient increase in the red count due both to the increased blood-pressure and to the concentration of the blood through the loss of water by perspiration. Physical exercise, taken to the point of producing fatigue, may produce a marked diminution in the number of red cells, due probably to the fact that regeneration of new cells cannot keep up with the destruction of the old cells. Passive exercise, in the form of massage, has a transient influence in producing an increase in the number of the red cells, owing to its increasing the general circulatory tone.⁴

¹ See Cohnheim, Kreglinger, Tobler, and Weber, *Ztschr. f. physiol. Chem.*, 1912, LXXVIII, 62; Schneider and Havens, *Am. Jour. Physiol.*, 1915, XXXVI, 380; Dallwig, Kolls and Loevenhart (*Am. Jour. Physiol.*, 1915, XXXIX, 77) believe that a decrease in the oxygen tension of the respired air stimulates the bone marrow to increased production of erythrocytes and hemoglobin. See Ocaranza, *Gaceta Med. de Mex.*, 1919, I, 157; Gregg, Lutz, and Schneider, *Am. Jour. Physiol.*, 1919, L, 216.

² Chamberlain has, however, shown (*Philippine Jour. Sc.*, Sec. B., 1911, VI, 467) that the average number of red cells in the tropics is little different from that in temperate zones. See Laquer, *Deutsch. Arch. f. klin. Med.*, 1913, CX, 189; Cohnheim and Weber, *Ibid.*, 225; and Lope, *Semana Med.*, 1913, XX, 1077.

³ See Hopmann, *Arch. f. Verdauungskr.*, 1913, XIX, 456.

⁴ See Hawk, *Am. Jour. Physiol.*, 1904, X, 384; Schneider and Havens, *Ibid.*, 1915, XXXVI, 239.

(6) Baths.

Careful investigation of the influences of both cold and hot baths has shown that an increase in the number of reds occurs under the action of these two conditions. The increase as a result of a cold bath may be as great as 2,000,000 cells, due to capillary stasis as a result of vasomotor constriction. Likewise, a hot bath will increase the number of cells by causing dilatation of the peripheral vessels and a consequent increase in the amount of blood at the point from which the specimen is taken. When marked perspiration follows either a hot or cold bath the blood becomes concentrated and, as a result, an increase in the number of red cells will obtain.

(7) Therapeutic Measures.

Any drugs which cause rapid loss of fluid from the body, as for instance emetics, purgatives, diuretics, and diaphoretics, will cause concentration of the blood and hence a coincident increase in the number of cells, providing the change is sufficiently rapid and the blood examination is made before compensation occurs. Among the drugs which increase the number of red cells we find iron and arsenic, both of which are particularly valuable in anemic conditions, the former in chlorosis and the latter in pernicious anemia. The compounds of mercury and of lead, on the other hand, have a destructive action upon the red cells, so that we find these drugs causing a diminution in the number of cells.

Pathologic Variations.

(1) Oligocythemia.

This is a condition characterized by a diminution in the number of red cells. It is usually associated with a decrease in the percentage of hemoglobin and with a slight reduction in the total volume of blood, although this latter factor is not invariably present. This condition is found, to a more or less degree, in all forms of anemia and may be temporary or permanent. The extent of the decrease in the number of red cells varies from 500,000 cells to a reduction of 4,000,000. This diminution in the number of cells is usually an indication of the severity of the anemia, the most marked decrease being observed in the pernicious types, Osler reporting probably the lowest count recorded, namely, 100,000 red cells.

While this oligocythemia is usually associated with oligochromemia, yet we find in chlorosis that the diminution of red cells is not as marked as is the reduction in the amount of hemoglobin, the color index in this condition being usually low. In some cases of chlorosis, however, the oligochromemia may keep pace with the oligocythemia. In pernicious anemia, on the other hand, we have a marked reduction in the number of cells and coincidentally a large decrease in the amount of hemoglobin, the result being that we have a very high color index as the usual sign of this condition. Cases showing the loss of a large amount of blood, as the result of hemorrhage have naturally an oligocythemia, but the percentage of hemoglobin may not necessarily be reduced to any great extent. This gives rise to the condition of secondary anemia, in which the color index may be high. In these cases of hemorrhage

a sudden reduction to the point of 1,000,000 or less cells is usually followed by a fatal result, although even here recovery has been made possible by rapid infusion of salt solution and compensatory activity of the blood-forming organs. In leukemia we find that the red cells are not usually diminished to a very great extent, the oligocythemia being generally more marked in the lymphatic than in the myeloid variety. Occasionally, however, we do find very low counts, in both varieties of leukemia. These same statements apply to splenic anemia, although the count here very rarely reaches a lower point than 2,500,000 cells.

A large number of conditions, aside from direct blood diseases, cause a diminution in the number of these red cells. Thus we find that the toxins of certain of the specific fevers, such as typhoid and pneumonia, may cause a marked anemia, but this is not necessarily the rule. Acute infection with pus organisms is frequently observed to cause an extreme and rapidly progressive anemia, the destruction of the red cells being in some cases very extensive. In malignant disease we usually find, especially if cachexia is present, a very extensive anemia which may lead to a diagnosis of the pernicious type. It is to be remembered, however, that a secondary anemia may assume all of the characteristics of the primary pernicious type.

(2) Polycythemia.

This is a condition characterized by an increase in the number of red cells and is sometimes called polyglobulia.¹ Whether this increase is actual and permanent or whether it be simply apparent, due to concentration of the blood or to unequal distribution of blood in the peripheral vessels, is still an unsettled question. It is true that many physiological causes previously enumerated do bring on a polycythemia, so that these should be remembered whenever a blood count is to be made. From the pathologic standpoint we find an increase in the number of red cells occasionally following active blood regeneration after hemorrhage. The number of corpuscles existing at any moment in the blood must represent a balance between factors of formation and destruction of these cells. Herrnhiser believes that the erythrocytes, formed without excessive function in bone marrow, may, somehow, be protected from the usual destruction, in which case polycythemia occurs. Likewise, we observe an increase in the number of cells in phosphorus poisoning, acute yellow atrophy of the liver, and in certain cases of general hepatic insufficiency. Why such an increase of cells occurs in these conditions is very hard to say. It has been found that the solids of the plasma are not increased, so that we are not warranted in assuming a concentration of the fluid elements. A polycythemia of uncertain origin is also seen in cases of poisoning with carbon monoxid and illuminating gas.²

In a condition called by Osler autotoxic enterogenous cyanosis, which is characterized by a marked increase in the number of red cells and also by enlargement of the spleen, we have reason to assume that a direct real increase

¹ Also erythremia.

² See Karasek, *Trans. Chic. Pathol. Soc.*, 1911, VIII, 173; Lipsitz and Cross (*Arch. Int. Med.*, 1917, XX, 889) and Lipsitz, Fuerth and Cross (*Ibid.*, 913) report a count of 10 million reds in a case of poisoning with cantharides.

in the number of red cells occurs, but the pathogenesis of the condition is uncertain. It has been traced to improper aeration of the blood and occurs also in congenital heart disease, mitral lesions in the adult, and in pneumonia and acute miliary tuberculosis. While this high blood count must be definitely admitted, its importance from a clinical standpoint is still unsettled. It is uncertain whether the rapid increase in the number of reds is due to an attempt on the part of the blood-forming organs to overcome the influences of the toxic substances upon proper oxidation in the system; yet it is certain that relief very frequently follows such an increase in the number of red cells, although in Osler's disease or, as it is called by some, Vaquez's disease, death frequently follows without any relief from the condition.¹

(E) Staining Properties of the Red Cells.

The normal red cell, like all living cells, is incapable of being stained with anilin dyes; that is, it is achromatophilic. Previous to staining, fixation of the cell must take place, the protoplasm being killed in this process. The normal fixed red cell has a marked affinity for various dyes of the acid type, such as eosin, orange-G, acid fuchsin, and indulin, and is therefore called acidophilic or oxyphilic. This cell normally takes up but one color from a mixture of dyes, and is called, therefore, monochromatophilic.

Polychromatophilia.

Under various pathologic conditions we find red cells which show a tendency to take up the basic stains, and for this reason are called basophilic or polychromatophilic cells. The tint of such abnormal red cells varies from that of a light indefinite shade of the basic stain to a dark distinct tone. Just what factors are at the bottom of this change, which is called polychromatophilia or polychromasia, is difficult to say. The normal acidophilic tendencies of the cell are due to the presence of hemoglobin and, in consequence, the normal staining properties will depend upon the relative richness of the cell in this pigment. As hemoglobin is always acidophilic, we cannot assume that the polychromatophilic properties of the abnormal red cells are due to variations in the hemoglobin, but must content ourselves with the belief that these changes have something to do with the protoplasm of the cell.²

This condition, which has been termed by Ehrlich anemic degeneration, is characterized by a diffuse basic staining property of the red cells. These basophilic cells are somewhat larger than the normal ones, show less biconcavity, and often are abnormal in shape. The megaloblasts are practically

¹ See White Lancet, 1912, XCII, 7; Lucas, Arch. Int. Med., 1912, X, 597; Cahn, Inaug. Dissert., Berlin, 1912; Chauffard and Troisier, Presse méd., 1913, XXI, 653; Friedman, Med. Record, 1913, LXXIV, 701; and Moewes., Deutsch. Arch. f. klin. Med., CXI, 281; Hertz and Ehrlich, Deutsch. Arch. f. klin. Med., 1914, CXVI, 43; Mosse, Ztschr. f. klin. Med., 1914, LXXIX, 431; Lamson, Jour. Pharmacol. and Exper. Therap., 1915, VII, 169; Lamson, Jour. Pharm. and Exper. Ther., 1915, VII, 169; Ibid., 1916, VIII, 167; Lamson and Keith, Ibid., 247; Freund and Rexford, Arch. Int. Med., 1916, XVII, 415; Pickard, Jour. A. M. A., 1916, LXVII, 1845; Lankhout, Nederl. Tijdschr. v. Geneesk., 1917, I, 294; Goyena and Masoch, Sem., Méd., 1919, XXVI, 113; Herrnheiser, Deutsch. Arch. f. klin. Med., 1919, CXXX, 315; Thaysen, Ugesk. f. Laeger, 1920, LXXXII, 473 and 514; Herman and Lyon, Ann. Surg., 1921, LXXIII, 223; Gitlow, Med. Record, 1921, XCIX, 227; Rydgaard, Hospitalstid., 1921, LXIV, 379 and 385; Pollock, Jour. Am. Med. Assoc., 1922, LXXXVIII, 724.

² See Montagnani, Sperimentale, 1919, LXXIII, 21.

always polychromatophilic and the normal erythroblasts of the bone-marrow usually show this degeneration. This fact would seem to point to the probability of this condition being a sign of regeneration of the blood as the erythroblasts are more prone to be polychromatophilic the younger they are.

Two distinct forms of polychromasia have been found. The first of these, known as the *polychromatophilic degeneration of Gabritschewsky*, is the diffuse basophilic staining of the cells which is found in various forms of anemia and extensively in the cells of the normal bone-marrow. The second of these, the *polychromasia of Maragliano*, is shown in severe anemias and in toxemias and appears as a more punctate basophilia, being closely related to the basophilic degeneration of Grawitz, which will be discussed later. While the polychromatophilia of the diffuse type is considered by Ehrlich as evidence of a degenerative process, that is as a coagulation necrosis of the discoplasm in consequence of which this takes up the albuminous principles of the plasma while it loses its power of retaining hemoglobin, yet there are evidences showing that this may be a regenerative process.¹ Polychromatophilia is often seen in cells which are undergoing degeneration, especially those following malignant disease, eruptive fevers, malaria, and after various poisons, such as that of snake venom.

The forms of partial polychromatophilia, which have been described under various names, such as vacuolization, pseudonucleation, globular decolorization, and more commonly Maragliano's endoglobular degeneration, are seen in normal blood in from 30 to 70 minutes after the specimen is made. They are usually found in the center of the cell, but may be near the periphery. There may be several such areas in a single cell, but the more common form is the single area of degeneration, which is usually round, may be elliptical, and may resemble a vacuole. These are shown quite distinctly in the unstained specimen and in this condition very frequently are seen in rapid motion. This motion is not of the true ameboid type, but is due more to the gradually progressing coagulation and consequent constriction of the protoplasm. These areas, whether in the fresh or stained specimens, may be mistaken for malarial parasites, so that the worker should be on his guard lest he make a wrong diagnosis without sufficient evidence. They differ in size from the parasites, and on focusing the specimen are characterized, usually, by a change from a smaller to a larger form, which variation is not evident with the malarial organism. The longer one searches in the unstained specimen for malarial organisms the more apt a mistake in diagnosis is to be made, as these "Maraglianos" develop quite rapidly. In the stained specimen these areas of coagulation necrosis will show a basophilic staining quality, while the chromatin element of the malarial organism will differentiate this body from the more usual degenerative area.

(F) Degenerations.

While the preceding conditions of polychromatophilia are to a certain extent true degenerations, yet they are more properly grouped under the heading of atypical staining reactions of the red cell.

¹ See Pfuhl, *Ztschr. f. klin. Med.*, 1913, LXXVIII, 102.

Basophilic Degeneration of the Red Cells.

This condition is distinct from the polychromatophilia above described and is known as the *punctate basophilia of Grawitz*. It is characterized by the appearance, in the body of the red cell, of granules of varying size, which stain with the basic dyes. The cell may be dotted throughout with these granules or may show this degeneration only in parts. These granules are not observed in the fresh unstained specimen and are not increased, as are the Maragliano areas, by allowing the blood to stand. These cells are observed in pernicious anemias, leukemia, in the toxemia of malignant states and especially in cases of lead-poisoning.

The size of the granules may vary from small dots to large granules, showing a diameter of one or more microns. Their origin is much in doubt, but they are generally considered to be areas of coagulation necrosis associated with either degeneration or regeneration of the red cells. They are quite distinct from the granular basic "stippling" observed in malarial conditions, but are probably evidences of direct toxic effects upon the protoplasm of the red cells. Their greatest clinical importance is probably in lead-poisoning, where they may be the only signs of abnormality in the blood picture. They may vary in number from day to day, showing in some cases five or six in one microscopic field, but, as a rule, they are present in much fewer numbers, as one observes them only after examining several visual fields. As a rule, they appear very early in cases of lead-poisoning, in one case observed by the writer within three days, and they may be present in the blood of a lead worker for several years after his exposure to the effects of the lead. They are usually the first sign of anemic change and usually persist longer than do the other abnormalities of the blood.¹

Ring Bodies.

Occasionally one observes in the red cells curious ring-like bodies which are in shape very much like the hyaline malarial ring with a circular refractive center. They change their shape in a peculiar way, much resembling the undulatory movements of the hyaline body. They do not increase in number or grow larger on standing as do the Maraglianos and they are observed in a large number of conditions, such as measles, pernicious anemia, and severe secondary anemia. Two types of these ring bodies must be distinguished, the first is usually more peripherally situated, occasionally has a definite crescentic shape or has an appearance much resembling that of a Maragliano, and is found especially in cases of measles. This finding has lead to the

¹ See Petry, *Biochem. Ztschr.*, 1912, XXXVIII, 92; Anders, *Jour. Am. Med. Assn.*, 1914, LXII, 1164; Linenthal, *Ibid.*, 1796; Schnitter, *Deutsch. Arch. f. klin. Med.*, 1915, CXVII, 127; Moller, *Hospitaltid.*, 1915, LVIII, 1287 and 1303. Walterhöfer (*Deutsch. med. Wchnsehr.*, 1920, XLVI, 116) believes that the pathologic significance of inclusion bodies lies not so much in their presence as the fact that erythrocytes containing them appear in the peripheral circulation. No matter how much the shape and staining properties of these inclusions may vary, they are always traceable to some reaction in the bone marrow, with exaggerated production of new red cells. It is only, he states, in those new red cells that the azurophil inclusions are found. The different forms of these bodies represent merely different stages in the process of extrusion of the nucleus. See, also, Renaud, *Bull. de la Soc. Méd. des Hôp. de Paris*, 1920, XLIV, 982; Bockhorn, *Ztschr. f. klin. Med.*, 1920, LXXXIX, 304.

assumption that these bodies were the organisms causing measles. The second form has been described by Cabot¹ and more recently by Schleip² in cases of pernicious anemia, leukemia, and secondary anemias. These latter bodies are larger than the former, more distinct, irregular in shape, frequently forming figure-eight bodies and are usually stained bright red with Wright's stain, but may occasionally show a blue shading. Herrick³ has observed thin, elongated, sickle-shaped red cells, some of them nucleated, in a case of severe anemia.

Various other forms of degeneration of the red cells occur, as for instance the appearance of rod-like areas resembling bacilli,⁴ which may keep up a constant vibratory motion carrying them through the entire substance of the cell. This finding should not confuse one in making a diagnosis of the presence of bacteria. Another form of degeneration, known as Ehrlich's hemoglobinemic degeneration, has the appearance of a small dark cell lying upon a larger paler one. These are probably areas of condensed protoplasm with the hemoglobin distinctly separated from the stroma. Such cells appear, occasionally, in certain types of malaria and are shown as corpuscles in which the hemoglobin is apparently condensed around the parasite. This degeneration is occasionally seen in nucleated red cells and may give the appearance of a microblast lying upon a macrocyte. It is best seen in cases of pernicious anemia and may explain some of the "acidophilic granules" of the red cells which have been described (Emerson).

(G) Isotonicity and Resistance of the Red Cells.

Normally, the relations of the cellular to the fluid portions of the blood are such that the hemoglobin and other constituents of the red cells are held intact within the limiting membrane of the cell. Any change in the osmotic pressure of the plasma is certain to be manifested by variations both in size and in composition of the cellular elements. We find, therefore, as previously discussed under the heading of Osmotic Pressure, that the inorganic constituents of the plasma are the factors upon which the volume of the red cell depends to a large extent.

By an isotonic solution, as applied to the blood, we mean a solution of such a strength as to preserve the corpuscles and to prevent passage of water, salts, and organic bodies from the plasma into the corpuscle or from the corpuscle into the plasma. In other words, an isotonic solution is one whose osmotic pressure is equal to that of the contents of the red cell. While the limiting structure of the red cell is indefinitely known, it seems to have certain properties which would lead us to assume that it is a semipermeable membrane, that is one which will permit the passage of organic compounds or of salts or their ions in both directions. This apparent selective activity is dependent both upon the laws of osmosis and diffusion through a semiper-

¹ Jour. Med. Research, 1903, IX, 15.

² Deutsch. Archiv. f. klin. Med., 1907, XCI, 449.

³ Arch. Int. Med., 1910, VI, 175. See, also, Bishop, Arch. Int. Med., 1914, XIV, 388; Cook and Meyer, *Ibid.*, 1915, XVI, 644; Pisani, Folia Hemat., 1915, XIX (1), 119; Emmel, Arch. Int. Med., 1917, XX, 586; Knoll, Deutsch. Arch. f. klin. Med., 1921, CXXVI, 237.

⁴ See Roth, Ztschr. f. klin. Med., 1912, LXXVI, 23.

PLATE XIX.



W. H. H. H. H. H.

RING BODIES IN RED CELLS. (AFTER SCHLEIP.)

meable membrane and upon the basic ideas of Ehrlich's side-chain theory. While other figures have been used at various times to represent the strength of a solution isotonic with the red blood-corpuscle, the one which seems more nearly to duplicate the actual condition is a 0.9 per cent. solution of sodium chlorid. This is or should be the normal salt solution which is used in physiological work and in transfusion. Loeb has shown that a properly balanced solution containing the chlorids of sodium, potassium, and calcium more nearly represents a proper "physiological salt solution" than does the simple 0.9 per cent. sodium chlorid solution. Such an isotonic solution will preserve the corpuscles, will prevent the passage of hemoglobin from the cells into the plasma, and will not permit of shrinkage or swelling of the cells.¹ Solutions which are stronger than the one above mentioned are called hypertonic solutions, while those of a lower concentration are termed hypotonic solutions. In the first of these shrinkage of the cells will occur, while in the latter phenomena of swelling will be observed.

The erythrocyte normally shows a certain amount of resistance to variations in the osmotic pressure of the blood and to the hemolyzing effects of various substances. It is true that the osmotic pressure of the plasma may be raised by the introduction of certain substances into the blood without causing phenomena of shrinking, swelling, or hemolysis to occur, yet we must not assume that the cell is not influenced by such changes in osmotic pressure. Simple variations in osmotic pressure come more nearly to the foreground in the explanation of these changes, especially of those occurring in most disease processes of a chronic type, while in the more acute infectious types associated with toxemia the biologic theory must be invoked. As yet little of value has been forthcoming from the study of the resistance of the blood-cells to variation in osmotic pressure, as the scope of the examination has not been sufficiently widened by application of accurate experimental methods. The writer is forced to say, from the results of his own experiments, as well as those of others with the more usual method of Hamburger, that no reliable data are at hand. It is highly probable that the little understood biologic influences are clinically much more important than are those due to variations in osmotic pressure, as this latter process cannot explain the hemolytic results of the toxins or of certain definite chemical compounds which bring about these results in concentrations which have little influence upon the tonicity of the blood.²

(H) Variations of the Red Cells in Childhood and in Old Age.

As previously stated, the average number of red cells is much higher than

¹ Ringer's solution is frequently used in this work. Its composition is: sodium chlorid, 9 grams; calcium chlorid, 0.24 gram; potassium chlorid, 0.42 gram; sodium bicarbonate, 0.2 gram; water, 1,000 c.c.

² See Pepper and Peet, *Arch. Int. Med.*, 1913, XII, 81; also, Gaisböck, *Deutsch. Arch. f. klin. Med.*, 1913, CX, 413; Holler, *Ztschr. f. klin. Med.*, 1914, LXXXI, 129; Höber, *Deutsch med. Wchnschr.*, 1915, XLI, 273; Roccavilla, *Rif. Med.*, 1915, XXXI, 813 and 873; Hamburger, *Biochem. Ztschr.*, 1915, LXXI, 464; Hill, *Arch. Int. Med.*, 1915, XVI, 809; Loeb, *Jour. Biol. Chem.*, 1916, XXVII, 339, 353, and 363; Hamburger, *Wien. Med. Wchnschr.*, 1916, LXVI, 521 and 575; Davis, *Ill. Med. Jour.*, 1916, XXIX, 280; Musser and Krumhaar, *Jour. A. M. A.*, 1916, LXVII, 1894; Neilson and Wheelon, *Jour. Lab. and Clin. Med.*, 1921, VI, 454, 487 & 568; Brahmachari and Sen, *Biochem. Jour.*, 1921, XV, 463; Fenn, *Jour. Exp. Med.*, 1922, XXXV, 271.

the normal figure at birth, gradually decreasing until the age of puberty when a gradual increase occurs again until middle life, after which a steady decrease is observed. It is to be expected that variations in the color tones (hemoglobin content) of these cells will vary normally with the age of the subject, being very high in the early days of life, gradually diminishing as puberty approaches, increasing until the middle periods of life, and steadily declining as the age increases. Aside from the number of cells, the blood in childhood shows little variation in the characteristics of the red cells, but the variations in the plasma are more or less marked, as the synthetic and retrograde products of tissue metabolism are much more evident in the period of development than during the middle periods of life.

As the subject becomes older the blood is laden with products of retrograde tissue change and the blood-forming organs become less active. It must be remembered that the various organs of the body contribute certain factors to the blood and may, therefore, all be considered as blood-forming organs in a definite sense. The ductless glands, in particular, pour into the blood certain substances which markedly influence the proper correlation of these various organs. Just what these substances are is not well established, so that the introduction of the word "hormone" by Starling does not at present have a specific meaning. As old age approaches variations become evident in the plasma, and through such influences changes in the morphology of the red cells are more or less frequent. Besides these plasma changes, gradual atrophic degeneration of the blood-forming organs takes place, so that we may find red cells showing the various types of anemic degeneration and may even see nucleated red cells as an evidence of the attempt on the part of the bone-marrow to overcome the gradual destruction of cells. The more notable variations in the red cells are observed in their size, owing to the hydremic state which is almost always characteristic of the plasma in advanced periods of life, but associated diseased conditions will, of course, give their characteristic changes.

(I) Functions of the Red Blood-cells.

The only well-recognized function of these cells is their oxygen-carrying power, which is dependent upon their hemoglobin content. Other oxygen catalysts, such as oxidase, peroxidase, and catalase, are found more largely in the leucocytes and play a significant rôle in the function of the whole blood. These will be discussed later. As is well known, the fluid portions of the blood are much more influenced by the various organs of the body than are the cellular elements, although the red cells will naturally be affected by variations in the hematopoietic organs, such as the bone-marrow, liver, and spleen. Our knowledge regarding the variation in the plasma is so uncertain that we have been led to interpret the changes in the histological appearance of the blood as significant of certain general pathological conditions. It is true that in some cases we do have direct disease of the blood-forming organs and, in consequence, variations in the cellular structures are of diagnostic importance, yet in the large majority of cases the preliminary changes must be resident in the plasma, those in the cellular structure being simply inci-

dental. It may be, therefore, necessary to assume a functional activity on the part of the red cells to overcome biologic changes in the plasma and if so we must attempt to discover just what plasma changes we are to regard as significant of the various conditions shown by the red cells in each true blood disease.¹

(6) Leucocytes.

(A) Appearance.

In the fresh specimen the leucocytes appear as colorless, high refractive bodies, somewhat larger than the red cells, and showing a definite nucleus. These white cells show distinct ameboid movement in the fresh specimen by virtue of which they are able to surround a foreign body and enclose it within their own protoplasm. This is the well-known property of phagocytosis which is such an important factor in Metchnikoff's theory of immunity. In contradistinction to the red cells, these white cells show many variations both in size and in shape. As the relations between the nucleus and the cellular protoplasm is quite distinct in many of these forms, some writers have been led to classify the white cells according to the peculiarities of the nucleus. Other writers classify them into granular and nongranular forms, as many of these cells show distinct granulations which are more clearly differentiated in the stained specimens. As more or less confusion exists regarding these different variations, it seems more rational at present still to classify these white cells with Ehrlich, who combines both the nuclear and granular characteristics in his classification.²

(B) Leucocytes in Normal Blood.

(1) Lymphocytes.

The cells of this class which occur normally in the blood are of two kinds, (a) the small cell, about 5 to 8 microns in diameter and (b) large cells showing a diameter of from 8 to 10 microns. Both of these cells have a very large nucleus which is usually centrally located but may have an eccentric position. It may be compact or coarsely reticulated and is not always as strongly stained as is the surrounding cytoplasm. Nor is it as deeply stained as is the nucleus of the normoblast, although it is very rich in chromatin. The nucleus of the larger cell may show very irregular staining properties. The nuclei of both of these cells are usually circular in outline, but may show an oval or a kidney-shaped structure, in which at times may be seen a distinct nucleolus. The protoplasm of these cells is usually in the form of a narrow rim surrounding the nucleus and showing a strongly basophilic homogeneous cytoplasm. The older cells may at times show a difficult staining property, may even be acidophilic, and occasionally exhibit a net-like structure in which are observed a few granules scattered throughout the protoplasm of the cell. This granulation, which occurs in about one-third of the cells seems to have

¹ Rous and Robertson (*Jour. Exper. Med.*, 1917, XXV, 651 and 665) show that the normal fate of the erythrocytes, in those species in which phagocytosis is negligible, is to be fragmented and removed from the blood by the spleen, and, under exceptional circumstances, by the bone marrow.

² See Pappenheim, *Die Ergebnisse der inn. Med. u. d. Kinderheilkde.* 1913, VIII, 183; also, Hoxie, *Interstate Med. Jour.*, 1913, XX., 1049.

little clinical significance, except as a probable sign of age of the cell, this granular stage being regarded as the end-point of their development. These granules are probably not representatives of true granulation, but seem to be more referable to nodal points of the reticular structure of the protoplasm.¹

These cells usually constitute from 20 to 25 per cent. of the leucocytes, their absolute numbers being from 1,200 to 2,000 per cmm., but the relative proportion of the small and large form being unsettled. The larger types of these cells are more closely associated with increased functional activity of the lymphoid tissues and should probably be considered the older cell of the two. According to Pappenheim, the large lymphocyte represents the mother cell from which all other leucocytes as well as erythrocytes are indirectly developed as a result of heteroplastic differentiation. Variations in technic of preparing the slides will often lead to differences in size of these cells, the thinner preparations showing these cells as larger forms, while the thicker preparations present the smaller type of these cells.

These cells are beautifully colored by the hematoxylin-eosin stain and by the various modifications of the Romanowsky stain, although the boundary between the nucleus and the protoplasm is not always clearly outlined by these latter stains. The granules, which are occasionally found in these cells and which are in size between the α and the ϵ granules of Ehrlich, show most clearly in preparations stained by the Giemsa stain and appear distinctly azurophilic. These granules do not appear at all with the triple stain.

These cells are frequently increased and frequently diminished, the former condition being spoken of as lymphocytosis, the latter as lymphopenia. A relative increase of the number of these lymphocytes is regarded as more or less characteristic of typhoid fever, especially when associated with a diminution in the total number of white cells. In lymphatic leukemia these cells are present in large numbers and are indicative of marked disturbance in the lymphatic structures.

(2) Large Mononuclear Leucocytes.

These cells, which are supposed to be derived from the spleen and are called, therefore, splenocytes, are as a rule two or three times as large as a red cell (12 to 15 microns), and show a nucleus which may be large and round, but is more frequently oval in shape or may be indented, forming the so-called kidney type of nucleus. It is to be remembered that we may have both large and small types of this large mononuclear leucocyte, the smaller forms being distinguished from the lymphocyte by the relation of the protoplasm to the nucleus. These nuclei are usually eccentric in position and are not always sharply outlined, they are poor in chromatin, but are strongly basophilic, although less so than are the nuclei of the lymphocytes. The protoplasm of these cells is very abundant in relation to the size of the nucleus and is very clear, hyaline, and nongranular in appearance. This protoplasm is much less basophilic than is the nucleus and shows a very fine reticulum with nodal thickenings which are somewhat more strongly basophilic and may give

¹ See Pappenheimer, *Jour. Exper. Med.*, 1917, XXV, 633; *Ibid.*, XXVI, 163; McJunkin, *Arch. Int. Med.*, 1918, XXI, 50; Bergel, *Berl. klin. Wchnschr.*, 1910, LVI, 015.

DESCRIPTION OF PLATE XX.

(*Triacid Stain.*)

1, 2, 3, 4. Small Lymphocytes.

Contrast the faintly colored protoplasm of these cells in the triple stained specimen with their intensely basic protoplasm in the film stained with eosin and methylene-blue, 17 and 18. The cell body of 1 is invisible. Note the kidney-shaped nucleus in 4.

5, 6. Large Lymphocytes.

With this stain the nucleus reacts more strongly than the protoplasm; with eosin and methylene-blue (19, 20), on the contrary, the protoplasm is so deeply stained that the nucleus appears pale by contrast. This peculiarity is also observed in the smaller forms of lymphocytes.

7, 8. Transitional Forms.

Note the moderately basic and indented nucleus, and the almost hyaline non-granular protoplasm. Compare 8 with the myelocyte, 7, Plate IV, these cells differing chiefly in that the myelocyte contains neutrophile granules.

9, 10, 11. Polynuclear Neutrophiles.

These cells are characterized by a polymorphous or polynuclear nucleus, surrounded by a cell body filled with fine neutrophile granules. In 11 the nuclear structure is obviously separated into four parts; in 9 it is moderately, and in 10 markedly, polymorphous.

12, 13. Eosinophiles.

The nuclei are not unlike those of the polynuclear neutrophile, except that they are somewhat less convoluted, and poorer in chromatin, staining less intensely. The protoplasm is filled with coarse eosinophile granules, the characteristics of which are clearly illustrated by 13, a "fractured" eosinophile.

14. Eosinophilic Myelocyte.

Compare with 15.

15, 16. Myelocytes. (*Neutrophilic.*)

These cells are morphologically similar to 14, except that they contain neutrophile instead of eosinophile granules. Note that the granules of the myelocyte are identical with those of the polynuclear neutrophile. A dwarf form of myelocyte is represented by 16.

(*Eosin and Methylene-blue.*)

17, 18. Small Lymphocytes.

Note the narrow rim of pseudo-granular basic protoplasm surrounding the nucleus and the pale appearance of the latter.

19, 20. Large Lymphocytes.

Budding of the basic zone of protoplasm is represented by 20. Both of these cells belong to the same type as 5 and 6.

21, 22. Large Mononuclear Leucocytes.

Compared with 19 and 20, these cells have a decidedly less basic protoplasm, but a somewhat more basic nucleus. In the triple stained film these differences cannot be detected, so that they must be classed as large lymphocytes.

23. Transitional Form.

The distinction between this cell and 24 is not marked; the nucleus of the latter simply being somewhat more basic and convoluted.

24, 25, 26, 27. Polynuclear Neutrophiles.

With this stain these cells show a feebly acid protoplasm, and lack granules. Note that the more twisted the nucleus the deeper it is stained. Compare with 9, 10, and 11.

28, 29. Eosinophiles.

Compare with 12 and 13.

30. Eosinophilic Myelocyte.

Compare with 14.

31. Basophile. (*Finely granular.*)

This cell is characterized by the presence of exceedingly fine δ -granules, staining the *pure* color of the basic dye. The nucleus is markedly convoluted and deficient in chromatin. The cell here shown was found in normal blood.

32, 33, 34, 35, 36. Mast Cells.

The granules take a *modified* basic color, as shown by their royal-purple tint in this illustration. Note their unusually large size and ovoid shade in 35, their peculiar distribution in 35 and 36, and their irregularity in size in 32 and 36. With the triacid mixture these granules, as well as those of the finely granular basophile, 31, remain unstained, showing as dull-white stippled areas in the cell body. The nuclear chromatin of the mast cell is so delicate and so freely stained that it is barely visible. These cells were found in the blood of a case of spleno-medullary leukemia.

PLATE XX.



THE LEUCOCYTES.

(1-16, *Triacid Stain*; 17-36, *Eosin and Methylene-blue.*)

(E. F. FABER, *fec.*)

(From *Da Costa's "Clinical Hematology."*)

the appearance of granulation.¹ These cells should not, however, be regarded as granular cells.

Occasionally we find cells, derived from the above, which have been called by Ehrlich "transition forms," which are the largest of all the white cells.² The nucleus is pale, often deeply notched giving it usually the appearance of a saddle-bag, and shows the characteristics of the large mononuclear type, being distinguished from the polymorphous nucleus of the neutrophile cells by its greater thickness and by its diminished intensity of staining. The extensive granulation of the polymorphonuclear leucocyte should make any mistake in diagnosis impossible. The protoplasm of these transitional cells is very abundant and shows faint basophilic properties and may even have a few fine granules, which are neutrophilic in character, in the neighborhood of the nucleus. These two forms of cell constitute between 3 and 5 per cent. of the leucocytes, their absolute number varying between 200 and 400 per cmm. Pappenheim considers that these large mononuclear cells develop directly from the large lymphocytes and then pass into the transition forms, which are the final developmental types.

(3) Polymorphonuclear Cells.

(a) Polymorphonuclear Neutrophiles.

These cells, sometimes called the finely granular cells of Schultze, are from two to three times the size of the red cell (10 to 12 microns) in diameter, the size depending upon the extent to which the cell is flattened by pressure. These cells are always smaller than the large mononuclear type and quite small specimens are frequently found in cases of myeloid leukemia. They are the most sharply characterized cells of the blood, their protoplasm being relatively great and showing slightly acidophilic staining properties. The reticular portion of the protoplasm is very slightly basophilic, showing occasionally nodal thickening or granules when stained with the methylene blue dyes. Throughout this protoplasm are scattered fine dust-like granules, the ϵ granules of Ehrlich, which are not all of the same size and which stain with the neutral principle of the dyes. In some cases these granules may gradually diminish so that they may be apparently absent or at least undetected. The triple stain is the characteristic stain for these granules, showing them of a distinct lilac color; but various other acid dyes, such as eosin, give various tints to them, so that they are recognizable as distinctly reddish granules in specimens stained by any of the Romanowsky modifications. The nuclei are elongated and constricted and may appear in the form of a bent rod or a mass of interwoven fine fibers, showing a thin chromatin-rich structure, or a distinctly S-shaped formation with oval thickenings. Frequently these nuclear masses appear as if separated into two or more distinct nuclei, but as a rule these masses are connected by fine bands and should, therefore, be considered rather as polymorphous than as polynuclear. The nucleus shows a reticular structure with nodal thickenings and is very basophilic.

¹ Bergel (Deutsch. Arch. f. klin. Med., 1912, CVI, 47) has shown that these cells play a large rôle in the hemolysis and lipolysis noted after injection of cells and lipoids.

² See Evans, Arch. Int. Med., 1916, XVII, 1; Ibid., XVIII, 692.

philic. These forms constitute from 65 to 75 per cent. of the total number of cells, their absolute number averaging 5,000 per cmm., the percentage ranging from 20 to 40 per cent. in the first years of life when the lymphocytes are increased. When outside of the blood-vessels they form the usual pus-cells and are extremely active phagocytes. These cells are derived from the mononuclear neutrophilic myelocytes which are normal habitants of the bone-marrow. They run their course as such and are not transformed into other cells, their granules diminishing in pathological conditions or with age, such change being associated with degeneration in the nucleus.

Perinuclear Granulation.

Sometime ago Neusser reported a finding of basophilic granules in certain leucocytes, especially the mononuclear and polynuclear type. These granules surrounded the nucleus and even appeared attached to it, showing a variable size and being more or less refractive. He regarded these perinuclear granulations as characteristic of the uric acid diathesis, but the work of Fitcher and of Simon show that these granules are undoubtedly artefacts which can be produced by heating and by variation in the staining.

Arneth's Classification.

Arneth¹ in his study of the blood in health and disease has been led to classify the polymorphonuclear neutrophiles into five classes depending upon the number of nuclear lobes. His divisions seem to be fairly constant in health, but show great variation, especially in infectious conditions. His first subdivision, known as Class 1, is divided into (a) M cells, which are mononuclear forms identical with Ehrlich's myelocyte, (b) W cells, representing forms which show only a slightly indented nucleus, the indentation never extending beyond the middle of the nucleus; this cell forms what is called the metamyelocyte, (c) T cells, in which the indentation of the nucleus is deeper than in the W cell, but there is no distinct separation into isolated loops, this form constituting the true polymorphonuclear type. The first two varieties of Class 1 are usually seen only under abnormal conditions, although the W cell may be found to the extent of 0.2 per cent. in healthy conditions. The cells of Class 1 are usually present, according to Arneth, to the extent of 5 per cent., Simon giving this percentage as from 4 to 9. The second class of Arneth embraces cells with two distinct nuclear fragmentations and shows three subdivisions: (a) 2 K cells, neutrophiles whose nucleus consists of two round nuclear portions; (b) 2 S cells, neutrophiles whose nucleus consists of two distinct S-shaped forms; (c) 1 K 1 S cells, neutrophiles whose nucleus consists of one round nuclear portion and one S-shaped division. These cells of Class 2 constitute, according to Arneth, 35 per cent. of the total neutrophiles, while Simon's figures are 21 to 47 per cent. In Arneth's figures we find the 2 S cells forming about 23 per cent. of the total of this class. The third class has three nuclear divisions and is subdivided into four parts, as follows: (a) 3 K cells, (b) 3 S cells, (c) 2 K 1 S cells, (d) 2 S 1 K cells. Arneth gives the percentage of Class 3 as 41, the 2 K 1 S and the 2 S 1 K subdivisions,

¹ Jena, 1904.

each representing approximately 16 per cent. of the total of this class. Simon's figures for this class range from 33 to 48 per cent. The fourth class comprises cells with four nuclear divisions showing five subgroups, as follows: (a) 4 K cells, (b) 4 S cells, (c) 3 K 1 S cells, (d) 3 S 1 K and (e) 2 K 2 S cells. This class, from Arneth's figures, shows a percentage of 17, the 4 K, 3 K 1 S, and 2 K 2 S types being largely in excess of the other forms. The fifth class comprises cells with five or more nuclear subdivisions and may be arranged into five groups, this class representing about 2 per cent. of the total neutrophils. It is probable that these various classes represent the gradual development of the polymorphonuclear neutrophile, the older the cell the greater the tendency to reach Class 5, while in conditions associated with new formation of cells, as in infectious conditions, we find the percentages of the earlier classes being increased, that of the later ones diminished. Minor and Ringer¹ have recently emphasized the prognostic value of this method in pulmonary tuberculosis.

(b) Polymorphonuclear Eosinophiles.

These cells, sometimes called the coarsely granular cells of Schultze, are somewhat smaller than the preceding, varying in size from that of a lymphocyte to that of the neutrophile. Their protoplasm is usually somewhat less in amount than is that of the neutrophile and may not be distinct. It is filled with coarse, round, or slightly oval granules about one micron in diameter, which are very refractile and appear in the fresh specimen distinctly black, while in the stained smear they take up the acid portion of the dye and are called, therefore, acidophilic, oxyphilic, or eosinophilic cells.² These granules are the α granules of Ehrlich, and are sometimes associated with the β granules of Ehrlich which are about the same size or a little smaller than the α granules and take both the acid and the basic stains, although with the ordinary staining solutions they appear, like the α forms, stained with eosin. The nuclei of these cells are coarsely reticulated, are larger and thicker than those of the neutrophils, are usually bilobed, and more frequently show distinct separation of these lobes. These nuclei do not stain very deeply with the nuclear dyes, so that they may be rather indistinct. These cells are probably derived from the mononuclear eosinophile myelocytes of the bone-marrow and constitute from two to four per cent. of the total number of leucocytes, their average number being from 100 to 200 cells.

¹ Am. Jour. Med. Sc., 1911, CXLI, 638. See, also, Ringer, *Ibid.*, 1912, CXLIV, 561; Kramer, New York Med. Jour., 1913, XCVII, 1241; Cummings, Calif. State Jour. Med., 1913, XI, 286; Cooke, Brit. Jour. Tuberc., 1914, VIII, 211; Macfie, Ann. Trop. Med. and Parasitol., 1915, IX, 435; Jour. Trop. Med. and Hyg., 1916, XIX, 41; Kahn, Jour. Lab. and Clin. Med., 1916, I, 599; Burgess, *Ibid.*, 1917, II, 240; Treadgold, Lancet, 1920, I, 699.

² See Müller (Wien. klin. Wchnschr., 1913, XXVI, 1025) for a discussion of the chemical properties of these granules. Acton and Knowles (Indian Jour. Med. Research, 1914, I, 523) believe the Kulroff bodies of the bone-marrow to be forerunners of the eosinophile granules. See, also, Pappenheim, Folia Hæmat., 1914, XVIII, 224; Downey, *Ibid.*, 1915, XIX, 148; Photakis, Ztschr. f. exp. Path. u. Therap., 1915, XVII, 270. Liebreich (Schweiz. med. Wchnschr., 1921, LI, 275; "Le Sang in Vitre," Paris, 1921) states that all factors which prevent coagulation prevent the appearance of eosinophile cells and of Charcot-Leyden crystals. He shows that the eosinophile granules are really crystals, which are identical with the Charcot-Leyden crystals, and that it is by the crystallization, instantaneous and complete, of the crystalline material that a cell becomes an eosinophile in the morphological sense.

(c) Polymorphonuclear Basophiles.

These cells, frequently called mast cells, resemble the neutrophile cells in the fresh specimen, but show quite distinct characteristics in the stained form. Their protoplasm is much the same as that of the neutrophile and shows the same relation toward the nucleus which does not, however, so frequently form distinct lobes as does the nucleus of the neutrophile. The size of these cells averages about 10 microns, but shows a marked variation, being very small in myeloid leukemia. These cells are characterized by their granules, the γ granulation of Ehrlich, which are more irregular in size than are the neutrophile granules and are not so extensively scattered through the protoplasm. These granules show the peculiar property of metachromasia, being colored red with violet dyes and with the blue dyes violet, although with absolutely pure methylene blue they take a blue shading. In cases of myeloid leukemia these granules are particularly soluble in water and may, therefore, not be seen if aqueous stains are used, while in normal blood the granules are usually water-fast. These cells stain best with Ehrlich's dahlia stain, or Türk's iodine solution, and take a tone which is not strictly basophilic, but resembles more that of mucin, on which account they have been called mucinophiles. Whether these granules are all of the same significance is questionable. The true mast cell granules are known as the γ granules, while other basophilic granulations have been found by Ehrlich and are known as the delta (δ) granules.¹ These latter granules are found in the large mononuclear cells, especially in the lymphocytes, and do not stain with Ehrlich's dahlia stain. Whether these latter bodies are true granules or nodal thickenings is at present uncertain. True mast cells probably originate in bone-marrow from a granular mononuclear type corresponding to other types of myelocytes (Pappenheim). These cells constitute about $\frac{1}{2}$ per cent. of the total leucocytes, averaging between 0 and 50 per cmm.

Ehrlich's dahlia stain for these mast cells is made as follows:

Distilled water,	100 c.c.
Saturated absolute alcohol solution of dahlia,	50 c.c.
Glacial acetic acid,	10 c.c.

The specimens are heated or fixed by alcohol and are then stained in the above stain for 5 to 10 minutes, the mast cells appearing of a distinctly violet tone.

Türk's Iodine Method.

The specimens are fixed with heat and are then first stained in a 1 per cent. alcoholic methylene blue solution, warming the slide very carefully until it steams. It is then allowed to cool, washed quickly in water, and dried between filter-paper. The slide is then covered with a solution of iodine in potassium iodide of the strength 1:300. Allow this solution to act not longer than $\frac{1}{2}$ minute, pour off and mount in the following syrup:

¹ Huguenin (Zentralbl. f. allg. Path. u. path. Anat., 1912, XXIII, 725) reports the presence of sudanophile granules in the mast cells in an obscure case. See, Graham, Jour. Exp. Med., 1920, XXXI, 229.

Iodin,	1 gram.
Potassium iodid,	3 grams.
Distilled water,	100 grams.
Gum arabic q. s. to make a syrup.	

The mast-cell granules appear, when treated in this manner, very distinctly outlined and colored black. The nuclei are brownish in color, the erythrocytes yellowish-green, and the polychromatophilic erythrocytes dark green, the neutrophile and eosinophile granules faintly yellow.

(C) Leucocytes in Pathological Blood.

(1) Myelocytes.

Under this heading we must regard any cell of the bone-marrow as a myelocyte, but for diagnostic purposes we have reference more to mononuclear cells which are distinctly granular. While the granulations of these cells are usually either neutrophilic or eosinophilic, we may rarely find, especially in myelogenous leukemia, cells which show basophile granulations. The size of these myelocytes varies from that of the red blood-corpuscle to that of a large mononuclear cell.

(a) Neutrophile Myelocytes.

This myelocyte may be either large or small, in the first case being known as Cornil's myelocyte (amblyochromatic type), a cell which is much larger than the polymorphonuclear leucocyte (at least 15 microns in diameter), and showing a round, pale, eccentric nucleus which stains feebly but not diffusely. This cell shows many distinct neutrophile granulations and has a narrow zone of basophilic protoplasm surrounding the nucleus. It is found almost entirely in myelogenous leukemia and has been called by Pappenheim the heteroplasic promyelocyte. The second type of neutrophile myelocyte is known as the Ehrlich myelocyte (trachyochromatic type of Pappenheim) which is a medium-sized cell with a pale central nucleus which stains deeply but not diffusely. This cell shows extensive neutrophilic granulation of the protoplasm, which is faintly oxyphilic, and has a nucleus which is either perfectly round, oval, or indented, but is never lobed nor pycnotic. A distinction between the myelocyte and the polymorphonuclear leucocyte should be based entirely upon the structure of the nucleus, all those cells with round, oval, or kidney-shaped nuclei which occupy at least one-half of the cell and show neutrophile granulations but no diffuse staining of the nucleus must be called myelocytes. It is to be remembered, however, that this type develops into the polymorphonuclear neutrophile, so that in abnormal blood we may have all gradations between these two types.

(b) Eosinophile Myelocytes.

These cells are exactly analogous to the preceding, with the exception that the granules of the more mature form show distinct eosinophilic tendencies. The younger forms of these granules may show a purplish-violet or even blue color, owing, as Simon states, to the fact that the young eosinophilic granule is physically cyanophilic and chemically amphophilic, whereas the mature

granule is physically erythrophilic, but chemically absolutely oxyphilic. The size of these cells is more or less variable, so that it is probable that we have the two types of eosinophile myelocytes, corresponding to the large and small neutrophile myelocytes. These cells occur more frequently in leukemia, in association with tumors of the bone-marrow¹ and in the pseudoleukemic anemia of children.

(c) **Basophile Myelocytes.**

These myelocytes may be of variable size, but are characterized by the large centrally located nucleus, which is not clearly defined from that of the surrounding slightly basophilic protoplasm. The granules are distinctly basophilic and in some cases are very numerous, while in others they may be widely scattered through the protoplasm.² These cells are practically never seen except in cases of severe splenomyelogenous leukemia, in which they may reach as high as 47 per cent. with an absolute count of 140,000 cells (Taylor).

(2) **Irritation Forms.**

These cells vary in size from a lymphocyte to a large mononuclear cell, resembling more nearly the former. They are mononuclear, nongranular cells, thus differing from the myelocyte which is always granular. The nucleus is round and eccentrically placed, showing a very slight chromatin network and staining with the triple stain of a bluish-green color while with the Romanowsky dyes the color is a pale blue. The protoplasm is stained a deep brown with the triple stain and is thus differentiated from other forms of cells. With the methylene blue dyes the protoplasm appears more deeply stained than does the nucleus. These cells were first described by Türk and would seem to have the same significance as do the myelocytes, namely, an indication of marked activity of the bone-marrow. Pappenheim regards them as plasma cells and largely derived from the lymphocytes.

(3) **Degenerated Forms.**

Occasionally we find in normal blood degenerated leucocytes which stain poorly and show no granules. These may be even so much degenerated that they show as the so-called basket cells or "shadows." This condition is very frequently seen in severe infectious diseases, while in the acute leucocytoses which occur under many influences diminution in the number of neutrophilic granules as well as swelling and fragmentation of the bodies of the leucocytes is very common. The changes in the staining qualities of the nucleus seem to be the most significant of the lesions in the acute type of degeneration of the leucocytes. In chronic degeneration of the leucocytes we find hydropic degeneration, which is frequent in the blood of chlorosis and, when the nuclei are involved in this degeneration, seems to be limited to certain cases of leukemia. Besides such changes we find fatty degeneration as well as glycogenic degeneration. This fatty degeneration is characterized by the appearance of fat globules in the leucocytes which stain with osmic acid and with Sudan-III. For this latter reason they have been styled

¹ von Roznowski (Ztschr. f. klin. Med., 1915, LXXXI, 377) believes that the presence of myelocytes in large numbers in cases of cachexia points to metastasis in the bone-marrow.

² See Aubertin and Chabanier, Ann. de Méd., 1915, II, 399

PLATE XXI.



IODOPHILA.

sudanophiles, and have been very carefully studied by Buttini and Comesatti, as well as by Cesaris-Demel.

Iodophilia.

In many pathologic conditions, especially in acute infectious diseases and in those associated with all types of sepsis, a so-called glycogen reaction or iodophilia may be demonstrated in the bodies of the leucocytes, as well as in certain extracellular granules. The technic of this method is as follows: An unfixed dry blood smear is exposed to the vapor of solid iodine until it is stained a brownish color. After the specimen is stained it is mounted in the syrup described on page 603 and is examined with an oil-immersion lens. The blood of normal individuals, stained by this method, shows the protoplasm of the leucocytes of a bright yellow, while the nucleus takes on a much lighter tint. In pathologic blood two types of reaction can be noted. The intracellular one, which is of greater clinical importance, shows a more or less marked diffuse brown color of the entire protoplasm of the leucocyte, or the protoplasm contains reddish-brown granules which may be more or less distinct. The extracellular reaction is evident in the blood plates, while the intracellular type is more particularly confined to the neutrophils, although the mononuclear leucocytes may occasionally be tinged brown. Much difference of opinion exists as to the nature of this brown-staining substance, Ehrlich regarding it as glycogen, while Czerny considers it as an antecedent of amyloid, and Goldberger and Weiss regard it as peptone. Kaminer regards this reaction as a degenerative change and not as an evidence of regeneration. While this reaction has little value in differentiating infectious or septic conditions one from the other, it is sometimes of importance in making a diagnosis between purulent and nonpurulent affections, being present in the former and absent in the latter. These granules have been found by Hofbauer in pernicious anemia, secondary anemia, and leukemia, but not in chlorosis or pseudoleukemia. This reaction is observed in pneumonia, but is seldom seen in tuberculosis, typhoid fever and diphtheria. It is, however, not to be regarded as dependent upon infection, as it occasionally obtains in non-infectious conditions.¹

(D) Differential Counting of the Leucocytes.

By a differential counting of the leucocytes is meant the counting of the different varieties of the leucocytes found in the stained smear and the calculation of each type in terms of percentage. The technic of this method is that previously outlined and consists in making an even smear upon a glass slide and staining it with any of the stains previously mentioned, noting that the triple stain does not bring out the granulations of the leucocytes with the exception of those of the neutrophils.² It is self-evident that the larger the number of leucocytes counted the greater will be the possibility of arriving at true percentage relations. It is wise, therefore, to count at least 250 of

¹ See de Haan, *Biochem. Ztschr.*, 1922, CXXXVIII, 124.

² See Arneth, *Deutsch. Med. Wchnschr.*, 1913, XXXIX, 2560; also, Dunzelt, *Münch. Med. Wchnschr.*, 1913, LX, 2616.

these cells, and in many cases, to extend this to 500. If the smear is even and the leucocytes well distributed throughout, 100 cells will frequently suffice.

For a differential count a satisfactory classification is an absolute essential. As none of the systems at present advanced are entirely adequate, we still use the classification of Ehrlich, which is as follows: Small mononuclears, large mononuclears (including the transitional), polymorphonuclear neutrophiles, eosinophiles, basophiles (mast cells), and myelocytes. The characteristics of these cells have been previously given, but may be summed up in this connection. By a small mononuclear is meant any nongranular mononucleated cell smaller than a polymorphonuclear neutrophile. A large mononuclear is any nongranular cell with a round or oval nucleus and larger than a polymorphonuclear neutrophile. A cell of the same description and size but with an indented nucleus is a transitional form. The polymorphonuclears are cells which are about 10 microns in size and show a diffuse granulation, which may be either neutrophile, eosinophile, or basophile. It should be remembered that the mast cells appear as nongranular forms when the triple stain is used, so that the characteristics of the nucleus in its relation to the protoplasm must be borne in mind. The percentage relations of these cells in normal blood are as follows:¹ It is probable that the figures given are not really accurate at least for the routine cases in this country. The usual "normal" variations noted are a reduction in number of the neutrophiles and an increase in small mononuclears (lymphocytes).

	Percentage.	Number per cmm.
Small mononuclears,	20-25	1200-2000
Large mononuclears,	3-5	200-400
Polymorphonuclear neutrophiles,	65-75	5000
Polymorphonuclear eosinophiles,	2-4	100-200
Polymorphonuclear basophiles,	0-1/2	0-50

In the writer's laboratory differential staining is usually carried out with the use of the Wright or Giemsa stain as he has found the usual triple stain quite unreliable. Although the granular differentiation is not as distinct as could be desired, yet one soon becomes accustomed to the staining of various cells so that it is not a matter of great difficulty to distinguish the various types. In many cases it is not the easiest matter to distinguish myelocytes from the small mononuclear types, but careful study will usually clear up any obscurities which exist.

¹ See Bunting, *Am. Jour. Med. Sc.*, 1911, CXLII, 698; Galambos, *Folia Hæmat.*, 1912, XIII, 153; von Torday, *Virchow's Arch. f. path. Anat.*, 1913, CCXIII, 529; Mehrtens (*Arch. Int. Med.*, 1913, XII, 198) calls attention to the frequency of relatively low polymorphonuclear leucocyte counts with high lymphocyte counts in the routine clinical work. Warfield (*Jour. Am. Med. Assn.*, 1915, LXIV, 1296) advises subdivisions of these groups so that we have seven classes. Leo-Wolf, *Interstate Med. Jour.*, 1915, XXII, 1235; Gruner, *Brit. Jour. Surg.*, 1916, III, 506; De Boe, *Florida Med. Assoc. Jour.*, 1916, II, 204; Turban, *Ztschr. f. Turberk.*, 1917, XXVI, 242. Cardenal, (*Sig. Med.*, 1916, LXIII, 785) confirms the value of Sondern's line of resistance as applied to the differential count. See, also, *Jour. A. M. A.*, 1917, LXVIII, 730; Levy, *Texas State Jour. Med.*, 1917, XIII, 179; Blumberg, *Am. Jour. Syph.*, 1918, II, 734; McJunkin and Charlton, *Arch. Int. Med.*, 1918, XXII, 157.

(E) Number of Leucocytes.

The normal number of leucocytes in a cmm. of blood has been given various figures. As a rule, it may be said that anything above 10,000 leucocytes per cmm. should be considered pathological, the normal variation running from 5,000 to 9,000 cells. In estimating the normal number of white cells, both in health and in disease, a large number of factors which influence these cells must be taken into consideration. Thus vasomotor phenomena, variations in the volume of the plasma, inflammatory processes, state of digestion, age, variations in different parts of the circulatory system, and many different disease processes usually bring about an increase in this number, while many pathological conditions are associated with a reduction. An increase in the number of white cells is usually spoken of as a leucocytosis, but it must be remembered that such an increase may be purely physiological and should be sharply differentiated from a pathological increase which is clinically the more important. This increase is usually referable to the increase in the number of the polymorphonuclear neutrophiles, while an increase in the other varieties of cells is spoken of as a lymphocytosis, myelocytosis, eosinophilia or an eosinophilocytosis, or a mixed leucocytosis.

A diminution of the number of polynuclear neutrophiles is designated as a leucopenia, which is the more usual form in which reduction of the cells occurs. It is to be remembered that these conditions may be transitory and symptomatic pointing to a purely physiological process, while a more permanent and more marked increase or decrease in the number should be considered pathological. It is rare that we find the increase limited absolutely to one variety of cell, the increase in the others being less marked. The absolute number of these cells is much more to be regarded than their percentage relation, as with an increased number of leucocytes the actual number of some of these varieties may be increased, although the percentage may be diminished; while with a low leucocyte count the percentage may be increased and the absolute number diminished. It is wise, therefore, to report not only the percentage relations of the leucocytes, but also the actual number of these cells per cmm. This will correct mistaken ideas as to an apparent increase or decrease in any particular variety of cell. Thus a differential leucocyte count may show a percentage of 50 for the neutrophiles and at the same time an actual number of 10,000 per cmm., giving rise to confusion as to the actual relation of these important leucocytes.

Leucocytosis (Polymorphonuclear Neutrophilosis).

As stated above, anything above 10,000 leucocytes per cmm. should be regarded as a leucocytosis. This is, however, relative and should not be considered pathological without taking into consideration all of the physiological and pathological influences. Regarding the theoretical cause of leucocytosis, little is definitely known. The influence of infectious processes is such as usually to increase the number of leucocytes in the blood, as an attempt on the part of the system to overcome by phagocytosis the action of the bacteria of the various diseases. Yet we find in some of these infectious processes, notably in typhoid fever, a marked reduction in the number of the white cells,

although the bacillus typhosus is present in large numbers in the blood at the same time. We must, therefore, assume some specific influence upon phagocytosis and chemotaxis, as a general infection is not necessarily associated with an increase in the number of white cells, but is dependent more upon the specific nature of the infection. The work upon opsonins and vaccine therapy may open up an entirely new field in our study of this subject. The leucocytosis shown in noninfectious conditions is still a matter of much dispute and has probably more to do with variations in the plasma than with direct increase in the number of white cells.

Classification.

According to Limbeck,¹ the following classification of the various leucocytoses is the most comprehensive:

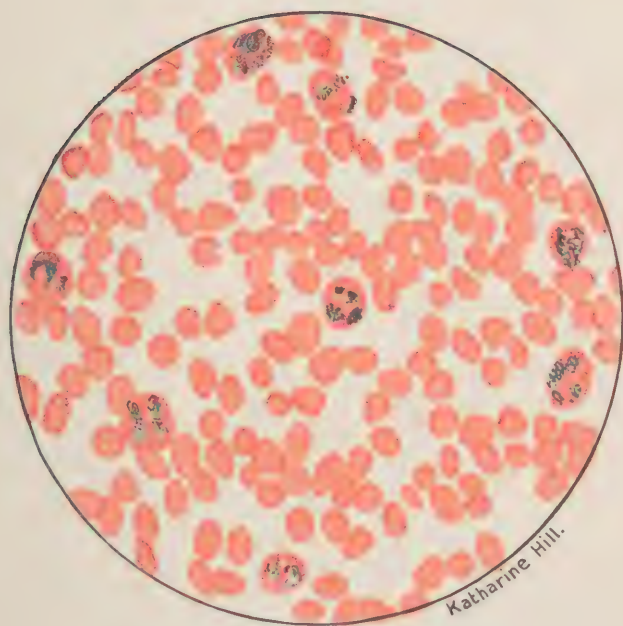
- (1) Physiological leucocytosis.
 - (a) Leucocytosis of digestion.
 - (b) Leucocytosis of pregnancy.
 - (c) Leucocytosis of the new-born.
 - (2) Pathological leucocytosis.
 - (a) Inflammatory leucocytosis.
 - (b) Leucocytosis associated with malignant tumors (cachectic leucocytosis).
 - (c) Posthemorrhagic leucocytosis.
 - (d) Agonal or antemortem leucocytosis.
 - (3) Leucocytosis following medicinal and therapeutic measures.
 - (4) Leucocytosis from other causes.
- (1) **Physiological Leucocytosis.**
 - (a) **Leucocytosis of Digestion.**

Normally a leucocytosis of the polynuclear type will be observed beginning about one hour after a meal rich in proteins and will reach its maximum in from three to five hours.² The actual figure reached varies in different persons, being usually an increase of about one-third, the maximum often reaching 15,000 cells, but more usually not much over 10,000. In this increase the small mononuclear cells may be absolutely as well as relatively increased. In some persons this leucocytosis of digestion does not appear, which fact may be referable to marked torpidity of the intestines, to a prolongation of the process of digestion, or to a large absorption of fluids. It has been found that a highly albuminous diet has a much more marked influence upon this leucocytosis than does a diet of vegetables and fat. The rapidity of absorption and of digestion must be taken into consideration as factors which influence the appearance or nonappearance of the leucocytosis. In children the increase in cells is much more marked than in the adult, due probably to the increased digestive and absorptive powers, providing the food taken is, as it should be, easily digestible and absorbable.

¹ Jena, 1896.

² See Brasch, *Ztschr. f. exp. Path. u. Therap.*, 1912, X, 381; also, Mitchell, *Am. Jour. Dis. Child.*, 1915, IX, 358. Mauriac and Cabouat (*Paris Méd.*, 1921, XI, 407) think it would be advisable to speak of the digestive fluctuation rather than of the digestion leucocytosis, as the counts may vary greatly within short periods.

PLATE XXII.



Katharine Hill.

POLYNUCLEAR LEUCOCYTOSIS. (WRIGHT'S STAIN.)

Various pathologic conditions influence this digestion leucocytosis. Thus Müller has found that in cases of carcinoma of the stomach a digestion leucocytosis is rarely observed after heavy meals. There are, however, a few cases of gastric cancer in which a slight increase has been observed. This failure of leucocytosis is probably due, as Schneyer shows, to lessened absorption as a result of involvement of the lymphatics rather than to a malignant stenosis of the pylorus, as the benign stenoses are usually associated with a leucocytosis. That the lack of leucocytosis is due more to diminished absorption than to lack of digestive power is shown by the usual occurrence of a digestion leucocytosis in ulcer of the stomach, chronic gastritis, and in dyspeptic conditions. Just why carcinoma, with or without stenosis, should be associated with a normal or subnormal number of leucocytes after digestion is hard to explain, in view of the fact that these other conditions are associated with a leucocytosis. Practically all absorption of food material occurs from the bowel and we would naturally expect to find disorders of the intestinal canal leading more frequently to a normal leucocyte count than to a leucocytosis. Very little detailed work has been done upon the influence of enteric troubles upon the leucocyte count, but the writer has seen several cases in which a digestion leucocytosis did not occur and in which the findings both ante- and postmortem were entirely related to the bowels. The examination of the blood may, however, clear up a diagnosis of carcinoma, but will not always permit of a differential diagnosis between this condition and pernicious anemia. It is to be remembered that a leucocyte count must be frequently made in order that this test may be of any value whatever and that a considerable rise only is to be taken as evidence of a leucocytosis. Patients with cancer either of the stomach or of other viscera frequently show a leucocytosis as the result of cachexia and this should be remembered in the interpretation of a leucocyte count following digestion.

(b) Leucocytosis of Pregnancy.

It has been shown that from 50 to 75 per cent. of cases of pregnancy are associated with a leucocytosis, averaging about 15,000 cells per cmm. This is especially true of primiparæ, but is often shown in the multiparæ. Just what cause can be given for this rise is uncertain. It is more than likely that a condition of slight intoxication is present due to the overloading of the blood of the patient with the products of metabolism of the fetus. These substances are not normal to the blood of the woman and in consequence act as foreign bodies which may attract, by their chemotactic influence, the white cells and bring about an increase. As these cases of pregnancy show an absence of a digestion leucocytosis it has been assumed that the increase in leucocytes is due to a prolonged digestion leucocytosis, but this does not seem probable. The changes in the breasts and in the uterus during this period have suggested these organs as the factors influencing this leucocytosis, but such inferences do not seem to be directly warrantable. The leucocytosis of pregnancy is a mixed leucocytosis, all of the various types of leucocytes, with the exception of the eosinophiles being increased. After the birth of the child the leucocytes gradually diminish in number and normally reach their usual

values in from four to fourteen days after delivery. Should complications, such as postpartum hemorrhage or septic fever arise, the leucocyte count may remain high until these complications have subsided. In multiparæ these changes in the number of leucocytes are not so marked as in the case of the primiparæ but a slight rise always occurs. This fact has been attributed to the lessened reactivity of the organisms to the influence of the toxic substances thrown into the blood from the cells of the fetus.¹

(c) Leucocytosis of the New-born.

As Askanazy has found, the blood of the fetus shows a diminution in the number of leucocytes, owing to the fact that there is no function as yet established for these cells *in utero*. In the blood of the new-born, however, a leucocytosis running from 15,000 to 20,000 cells may be observed, these figures going as high as 40,000 under the influence of the first feeding. As the weight of the child begins to diminish these cells are markedly reduced in number to about 8,000, and are subsequently increased to 10,000 as the child begins to gain in weight. The high leucocyte count at birth is more probably due to the rapid blood formation than to a concentration of the blood, although this latter factor as well as that of the influence of digestion must be taken into consideration. The increase in the number of cells is chiefly limited to that of the small mononuclear cell, the differential count of the leucocytes showing during the early periods of life from 40 to 60 per cent. of the total number. This leucocyte count, if taken at the moment of birth, will not, however, vary much from that of the adult, the change becoming more marked in the early periods of life.

(2) Pathological Leucocytosis.

(a) Inflammatory Leucocytosis.

In most cases of inflammatory nature, of acute infections and general febrile diseases, there is observed an absolute increase of the polymorphonuclear neutrophiles, which runs more or less parallel to the temperature. This increase may run from 10,000 to 50,000 cells and diminishes as the influence of the inflammatory process is diminished. In general it may be said that leucocytosis represents the reaction of the individual to the disease. A high count may mean a vigorous reaction to the infection; a low count may mean either a poor reaction and hence an unfavorable condition of the patient, or it may indicate a very mild degree of infection with a normal reactivity of the patient. It must be said that all diseases of an infectious nature are not necessarily associated with a leucocytosis. For instance, pneumonia shows a leucocytosis which runs parallel to the degree of virulence; measles, influenza, malaria, and tuberculosis are rarely if ever associated with a leucocytosis unless complications arise or the conditions become very severe.² In typhoid fever we usually find a leucopenia which, however,

¹ See Baer, Surg. Gyn. and Obs., 1916, XXIII, 567.

² See Hess, Am. Jour. Dis. Child., 1914, VII, 1; McDougall, Brit. Jour. Tuberc., 1917, X, 159; Govaerts, Presse Méd., 1917, XXV, 180; Forbes and Snyder, Jour. Lab. and Clin. Med., 1918, III, 758; Friedman, Am. Jour. Med. Sc., 1919, CLVIII, 545; Douglas, Bull. Johns Hopk. Hosp., 1919, XXX, 338.

is associated with a relative lymphocytosis; if complications such as perforation arise a leucocytosis may appear. Among the conditions causing leucocytosis are acute lobar pneumonia, the count running between 20,000 and 100,000, depending upon the severity of the infection and the degree of resistance against the infection. Acute articular rheumatism, diphtheria, acute cerebrospinal meningitis, follicular and suppurative tonsillitis, scarlet fever, mumps, rabies, erysipelas, ulcerative endocarditis, small-pox, cholera, general pus infections of the serous membranes and of the mucous membranes,¹ acute bronchitis, and many other conditions cause a leucocytosis of the polymorphonuclear type. The variations in these many conditions will be discussed under the Pathology of the Blood.

The exact cause of this leucocytosis following inflammatory processes is still a matter of much discussion. The bone-marrow has been shown to be markedly increased as regards cellular proliferation in the early stages of the inflammation and may be so markedly changed as to cause permanent derangement in the functions of this blood-forming organ. That leucocytosis has much to do with immunity both from the standpoint of phagocytosis and from that of Ehrlich's side-chain theory cannot be questioned.² A leucocytosis must represent the attempt on the part of nature to rid the blood and the system of the bacterial and toxic products of the disease. Whether phagocytosis as such or under the influence of various opsonins is the direct cause of immunity and of recovery from any specific infection must be left to a later chapter.

(b) Cachectic Leucocytosis.

This variety of leucocytosis is the least uncertain. The cases of carcinoma and of sarcoma show a leucocytosis which is not definite for the particular kind of cancer, but is more usual with the latter than with the former type. The leucocytosis is usually one of the polymorphonuclear type, but is frequently associated with an increase in the number of mononuclear cells. This leucocytosis has no direct relation with the situation of the tumor and is not always present in all cases of cancer. Whether this leucocytosis be due to an intercurrent infection is a question which must be left for more detailed work, but it would seem wise to accept Ewing's statement that a marked leucocytosis in the course of a cachexia, from tertiary syphilis, tuberculosis, nephritis, and in the majority of cases of carcinoma, should suggest a search for a complicating infection, while in the sarcomata a leucocytosis is much more common as a direct result of the disease.

(c) Posthemorrhagic Leucocytosis.

A well-marked leucocytosis, which may begin in from ten to fifteen minutes and may reach as high as 20,000 cells within an hour, has been often observed following extensive acute hemorrhages. The leucocytosis in these cases bears a general relation to the extent and rapidity of the loss of blood and usually

¹ See Smith, Surg. Gynec. and Obst., 1913, XVI, 403.

² Gay and Claypole (Arch. Int. Ned., 1914, XIV, 662) call attention to the specific hyperleucocytosis following inoculation with typhoid vaccines.

disappears or diminishes long before regeneration of the blood has occurred.¹ This leucocytosis is of the polymorphonuclear type and is referable rather to the sudden outflow of lymph which occurs as a compensation for the loss of fluid than to a new production of cells, as this latter process does not take place for some time. In hemorrhages which are slight and long-continued as in cases of gastric or intestinal ulcer, the duration of the leucocytosis as well as its extent is very brief. Stassano and Billou assume from their work that a hypoleucocytosis follows a severe hemorrhage while a true leucocytosis is observed after the loss of small quantities of blood. These findings seem to be rather doubtful, except where the hemorrhage has been so extensive as to cause death.

(d) **Antemortem Leucocytosis.**

This form of leucocytosis has been questioned, especially by Arneth, but there seems to be little doubt that such a leucocytosis may occur if death does not take place too rapidly. In some diseases the leucocytes do not fall in number, but in some a distinct rise is noted which has been attributed by Ehrlich to the accumulation of white cells along the periphery of the blood-vessels as a result of slowing or stasis of the circulation. This type of leucocytosis is usually of the polymorphonuclear variety and may support the view of Limbeck that antemortem leucocytosis, when it does occur, is the result of a terminal infection, although it cannot explain those cases which show a lymphocytosis rather than the ordinary leucocytosis. The character of the antemortem leucocytosis must depend largely upon the precedent condition and will be associated with antemortem dissemination of bacteria, antemortem hyperpyrexia, vasomotor paralysis, serous exudation, etc.

(3) **Leucocytosis Following Therapeutic Measures.**

(a) **Drugs.**

It has been found following administration of tonic drugs, ethereal oils, myrrh, turpentine, camphor, peppermint, quinin, and other drugs, that a leucocytosis of a more or less extent occurs, which is probably referable to the same cause as is digestion leucocytosis. Many of these drugs if applied locally for the purpose of counterirritation were shown to have the same effect. Extracts of tissues, especially those containing large amounts of nucleinic acid and of purin substances, produce an extensive leucocytosis, which fact has been taken advantage of in the administration of the nuclein substances as therapeutic remedies. In the case of those drugs which destroy the red cells, such as the coal-tar antipyretics, chlorates, and illuminating gas, a normal number of white cells is usually observed, although Simon wrongly states that a hyperleucocytosis follows the use of such drugs. After the prolonged use of chloroform or ether a polynuclear leucocytosis is generally observed which is usually of short duration.² In this connection it is well to remember that an increase in the number of leucocytes of 10,000 to 15,000

¹ See Levison, Jour. Am. Med. Assn., 1915, LXIV, 1294; Dold, Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1916, XXIX, 68.

² See Mann, Jour. A. M. A., 1916, LXVII, 172; Leake, Jour. Am. Med. Assoc., 1922, LXXVIII, 1687.

above the normal value of the individual should be regarded as evidence of infection if this increase is sustained for more than a few hours.

(b) **Baths.**

After a cold bath the leucocytes of the polynuclear variety have been shown to be increased from 100 to 300 per cent.¹ This is true only if the bath is of moderate duration, a prolonged cold bath taken to the point of exhaustion will diminish rather than raise the number of white cells. Experiments on the result of hot baths have shown just the reverse condition, namely, a hot bath of short duration produces a decrease, while one of long duration causes an increase in the number of white cells. Massage in itself following either a hot or cold bath tends to increase the number of leucocytes.

(c) **Exercise.**

Prolonged muscular exercise, as taken in the form of gymnasium work, as applied in the various therapeutic movement treatments, and also as given in the more violent athletic contests produces a rise in the number of leucocytes which is temporary and is characterized more by an increase in the number of polynuclear cells than of any other variety of the leucocytes.

(4) **Leucocytosis from Other Causes.**

Cyanosis, passive hyperemia obtained by the method of Bier, shock whether physical or mental, injection of various toxins, such as Koch's tuberculin, the various autovaccines, and injection of various organic principles, such as peptone, pus, organ extracts, etc., have all been shown to produce a polynuclear hyperleucocytosis, either by causing stasis of the circulation or by increasing the chemotactic power of the serum.² The exact explanation of chemotaxis is still in doubt and we find that some substances are positively and others negatively chemotactic, thus accounting for the lack of phagocytosis in certain conditions, such as pneumonia, although in these same conditions we may have a leucocytosis as a direct result of an infection. From the work of Rosenow we learn that the phagocytic action of the leucocytes in pneumonia is increased by the addition of an extract of the active pneumococci; whether this extract increases the chemotactic power of the bacteria in the blood or whether certain restraining influences are overcome is still unsettled. Future work upon phagocytosis and upon the factors governing increased opsonic power in the various infections may show us what causes are effective in such conditions.

In the previous discussion of leucocytosis we have had reference more to an increase in the number of polynuclear cells, this type being more properly called polynuclear hyperleucocytosis. It is very rare that this condition exists in the absolutely pure state.

Mixed Leucocytosis.

By this is meant an increase of both granular and of nongranular cells of various types, its more common interpretation, however, being a leucocy-

¹ See Rovighi and Secchi (*Rif. Med.*, 1914, XXX, 617; *Münch. Med. Wchnschr.*, 1914, LXI, 1721) for a discussion of leucocytosis following exposure to cold.

² See Veraguth and Seyderheim (*Münch. med. Wchnschr.*, 1914, LXI, 301) for the leucocytosis following use of electricity.

tosis characterized by an increase in the number of neutrophile myelocytes. This neutrophilic myelocytosis is best seen in leukemia in which the absolute number of myelocytes may reach 150,000, the increase in no other condition, according to Ehrlich, rising above 1,000 cells. The increase in the myelocytes in leukemia is not limited to those of the neutrophile variety, but is commonly associated with a marked increase in the number of eosinophile types as well as with an increase in the number of mast cells. In pernicious anemia we may also find a large increase in the number of these myelocytes. These cells when increased in number are clinically significant of exhaustion of the bone-marrow, but should never be interpreted in this way unless they remain while the leucocyte count is falling; in other words, they are significant only when constituting a large percentage of the leucocytes.

Lymphocytosis.

A relative or absolute increase in the number of lymphocytes is of frequent occurrence and has occasionally some significance. In judging of this condition the actual number of cells present as well as their numerical relations to the other varieties of leucocytes must be considered. The term lymphocytosis must always be applied to an absolute increase in the number of these cells, the normal number being between 1,200 and 1,500 per cmm. Physiologically we find this condition in infants and during a digestive leucocytosis in the adult.¹ The child shows at birth from 50 to 65 per cent. of these cells which percentage gradually diminishes, reaching the normal percentage (20-25 or even as high as 35) about the age of puberty.

Pathologically this condition is observed in poorly nourished children,² those showing the "constitutio lymphatica," rachitis, whooping-cough, gastro-intestinal disturbances in the child, cervical adenitis, splenic tumors, in most infectious diseases of children,³ and especially in lymphatic leukemia, in which as high as 90 per cent. of a largely increased leucocyte count is referable to the small lymphocytes. In the splenomyelogenous form of leukemia and following removal of the spleen we observe a steady increase in the number of lymphocytes continuing during the first year. It must be remembered that a leucocyte count may be low and yet a lymphocytosis exist. This is shown in typhoid fever, amebic dysentery, chlorosis, pernicious anemia, scurvy, and other conditions in which the leucocyte count is low, the granular cells being diminished in number, but the lymphocytes being increased.⁴

¹ Kaufmann, Jour. Am. Med. Assn., 1914, LXIII, 1104; Staines, James and Rosenberg (Arch. Int. Med., 1914, XIV, 376) report a lymphocytosis due to increased altitude. See Bunting and Huston (Jour. Exp. Med., 1921, XXXIII, 593) for a discussion of the fate of the lymphocyte in the body.

² See von Hoesslin, Münch. med. Wchnschr., 1913, LX, 1129 and 1206.

³ See Marchand, Deutsch. Arch. f. klin. Med., 1913, CX, 359; Marcovici, Folia Hæm., 1915, XX, 136.

⁴ Borchardt (Deutsch. Arch. f. klin. Med., 1912, CVI, 182) shows that a lymphocytosis obtains in a large majority of cases of disease of the thyroid, hypophysis and suprarenal gland. This is oftentimes associated with a leucopenia. Cabot (Am. Jour. Med. Sc., 1913, CXLV, 335) calls attention to the occasional occurrence of a lymphocytosis in infections usually associated with a polynucleosis. See, also, Huhle, Deutsch. Arch. f. klin. Med., 1914, CXIII, 455; Mayer and Gourdy, Sem. Méd., 1916, XXIII, 475, 513; Ibid., 1917, XXIV, 39 and 113; Bristol. A. M. A., 1919, LXXII, 1048. Daland (Jour. Am. Med. Assoc., 1921, LXXVII 1308) believes that a lymphocytosis is an important diagnostic sign of periapical dental infection.

In the congenital and also secondary acquired syphilis we find a lymphocytosis which must be referred to involvement of the lymph-glands. The worker must not be led into a mistaken interpretation of his blood-findings, as enlarged lymph-glands may give the same picture of lymphocytosis as is seen in Hodgkin's disease, chronic and acute lymphatic leukemia.

Eosinophilia.

By this is meant an absolute increase in the number of eosinophilic cells. The average number of these cells is between one and two hundred per cmm., hence the term eosinophilia should be limited to those cases showing counts something above 250. We find a physiological eosinophilia during childhood, the average increase being about 1 to 2 per cent. above the normal adult finding. No physiological relations have been established between eosinophilia and sex, pregnancy, menstruation, digestion, or old age; however racial distinctions are sometimes shown by eosinophilia, the natives of southern China showing between 15 and 20 per cent. of the leucocytes as eosinophiles.

Pathological Eosinophilia.

We observe variations in the number of eosinophiles in various affections of the bone-marrow. Thus in splenomyelogenous leukemia, we may find these cells increased as high as 30,000 per cmm. According to Ehrlich, a diagnosis of this form of leukemia is warranted only when we have an increase in the number of eosinophiles, but it cannot be doubted that cases are relatively frequent in which these cells are not increased. In sarcoma of the bone-marrow as well as in osteomyelitis and osteomalacia, we find these cells usually increased in number. In chlorotic conditions we may find the eosinophiles moderately increased, while in secondary anemias especially those following infection with parasites a marked eosinophilia may be observed.¹ After extirpation of the spleen as well as in cases of chronic splenic tumor we find an eosinophilia which may run from 10 to 40 per cent. higher than the normal, constituting as high as 40 per cent. of the total number of leucocytes.² Whether or not a pure disease of the lymph-glands is associated with the eosinophilia is unsettled, but if metastases have extended to the bone, an eosinophilia of marked degree is usual.

True bronchial asthma is always associated with an increase in the number of eosinophiles, one case of Billings showing 54 per cent. of the total leucocytes as eosinophiles. This relationship in asthma is of importance from a diagnostic standpoint as asthmatic attacks from other causes are not associated with an eosinophilia. It is true that in emphysema a marked eosinophilia does occur so that we may find, in cases in which this condition complicates a tuberculosis, that an eosinophilia obtains. Tuberculosis of the lungs or of other tissues does not show an eosinophilia unless complicated by emphysema, cachexia, or secondary infection.³

¹ Beifeld and Barnes, *Bull. Johns Hopk. Hosp.*, 1916, XXVII, 181; Grosso, *Rif. Méd.*, 1916, XXXII, 314 and 340; Klinkert, *Ztschr. f. klin. Med.*, 1920, LXXXIX, 156, 172 and 177.

² See Giffin, *Am. Jour. Med. Sc.*, 1919, CLVIII, 618.

³ See Arneth (*Deutsch. Arch. f. klin. Med.*, 1912, CVIII, 323), for the relations of the eosinophiles in pneumonia. Chuprin (*Med. Obozr.*, 1913, LXXIX, 17) believes that eosinophiles gradually increase in the blood in favorable cases of tuberculosis; also, Brösamlen, *Deutsch. Arch. f. klin. Med.*, 1914, CXV, 146; Peppard, *Journal-Lancet*, 1915, XXXV, 478.

A large number of skin diseases are associated with eosinophilia, the highest count being reported by Zappert in a case of pemphigus showing 4,800 cells per cmm. The occurrence of this condition in skin diseases depends not so much upon the nature of the lesion as upon its extent, intensity and lack of healing tendency. Many skin lesions are known to be produced by toxic agents which have special chemotactic influences over the eosinophile cells; this fact may account for the eosinophilia in such conditions. The most usual skin diseases showing this increase are pemphigus, eczema, psoriasis, urticaria, purpura, scleroderma, lupus, leprosy, herpes zoster, and general gouty affections.¹

It is a general rule that the infectious fevers are not associated with eosinophilia, but with the acute polynuclear leucocytosis.² In scarlet fever, however, we find that eosinophiles are frequently markedly increased, while in acute rheumatism and in malaria these cells are usually present in more or less increased numbers.³ In gonorrheal infections the eosinophile leucocytes are very frequently marked in the discharge in the early days of infection, diminishing in number as the number of neutrophiles increases, and increasing again as the discharge clears up. It is generally believed that the eosinophile cells in the blood are increased coincidently with their increase in the gonorrheal discharge. It has been shown that the eosinophiles are usually increased in all forms of ovarian disease with the exception of cancer. In syphilis a uniform increase of the eosinophiles has been observed, but some cases do not show such a regular finding.

Outside of the splenomyelogenous form of leukemia and the true bronchial asthma, infection with various parasites is accompanied by the most pronounced eosinophilia. Any parasite from the harmless pin-worm to the most malignant uncinaria may cause an eosinophilia (Emerson). This eosinophilia is not necessarily constant nor does its extent bear any relation to the severity of the infection. A differential diagnosis between typhoid fever and trichinosis is frequently possible on the basis of the marked eosinophilia in this latter condition,⁴ although eosinophilia does not always obtain here.

Mast-cell Leucocytosis.

In myelogenous leukemia we find these cells increased to a very large

¹ Lifschütz, *Monatsschr. f. Kinderhke.*, 1914, XII, 603; Aschenheim, *Ztschr., f. Kinderhke.*, 1914, X, 503; Putzig, *Ibid.*, 505; Engman and Davis, *Jour. Cutan. Dis.*, 1915, XXXIII, 73; Brösamlen and Zerb, *Deutsch. Arch. f. klin. Med.*, 1915, CXVIII, 163.

² Schlecht and Schwenker (*Deutsch. Arch. f. klin. Med.*, 1912, CVIII, 405) show the relationship of eosinophilia to anaphylaxis. See Herrick, *Arch. Int. Med.*, 1913, XI, 165; also, Ahl and Schittenhelm, *Ztschr. f. d. ges. exper. Med.*, 1913, I, 111; Steiger and Strobel, *Zentralbl. f. inn. Med.*, 1913, XXXIV, 1073. Leopold (*New York Med. Jour.*, 1914, C, 225) finds an eosinophilia in chorea. Besancon and Moreau, *Ann. de Med.*, 1914, II, 85; Johns, *New Orleans, Med. and Surg. Jour.*, 1914, LXVII, 453; Weinberg and Séguin, *Ann. de l'Inst. Pasteur*, 1914, XXIII, 470; Herrick, *Arch. Int. Med.*, 1914, XIII, 794; Marcovici, *Folia Hæm.*, 1915, XX, 133.

³ See Ebbell (*Norsk Mag. f. Lægevidensk.*, 1912, LXXIII, 617) for a very naïve theory of the relation of eosinophilia to the parasitic diseases. Legueu and Morel, *Jour. d'Urol.*, 1916, VI, 605, show the association of eosinophilia to enlarged prostate of non-malignant type.

⁴ Cooper (*Wisconsin Med. Jour.*, 1914, XII, 365) reports a case of trichinosis with 72 per cent. of eosinophiles. See Lamierre and Lantuéjoul, *Ann. de Méd.*, 1920, VIII, 409; Homme, *Arch. path.*, 1921, CCXXXIII, 11.

extent, often outnumbering the eosinophile cells. An increase of these cells is generally regarded as the sole isolated pathognomonic sign of this disease. Their increase may be as high as 20 per cent. of the leucocytes. These cells have been found in a few cases of cancer, tuberculosis, syphilis, and other lesions of the skin, while in bone disease complicated by septic infection a few reports of positive findings have been made.

Leucopenia.

This is a condition characterized by a reduction either in the total number of leucocytes or in one or more groups of leucocytes. The most usual condition showing a leucopenia is typhoid fever in which we find the polynuclear cells markedly diminished, thus lowering the leucocyte count, while the large mononuclear cells are relatively increased. Anything below 5,000 cells is regarded as a leucopenia. It must be considered in this connection that the typhoid leucopenia remains only when the disease is limited to the intestinal canal; when other organs become involved a leucocytosis supervenes. The count in typhoid fever may run as low as 2,000 cells, while in tuberculosis of the lymph-glands it may reach 500 cells. In cases of starvation or malnutrition the leucocyte count is always low and in chronic intoxication with the heavy metals, morphin, alcohol, and cocain, we observe a very low count, as a rule. A general rule to be observed in typhoid fever is that an increase in the number of white cells following a low leucocyte count is evidence of complications or of a mistaken diagnosis. It must be remembered that a relapse in typhoid may bring on a hyperleucocytosis, but this must occur during the afebrile period, otherwise a leucopenia will remain.¹ In measles, also, we observe a leucopenia following the eruption and a leucocytosis preceding the eruption. This leucopenia affects the polynuclear neutrophiles while the lymphocytes are relatively increased, with disappearance of the eosinophiles. In cases of uncomplicated influenza we usually find a diminution in the number of leucocytes, although a normal number may obtain. This enables one to differentiate influenza from pneumonia, in which latter condition a marked leucocytosis is practically always present.² In pernicious anemia and in splenic anemia we find a marked leucopenia during the active periods of the disease, the count running, as a rule, between two and three thousand cells.

In cases of hepatic insufficiency a leucopenia is frequently noted following a test ingestion of 200 grams of milk, as advocated by Widal. The leucopenia or "hemoclastic crisis" as it is styled may be noted within twenty minutes of the ingestion. In cases of diabetes, one may use 20 grams of glucose instead of the milk for testing the reaction on the leucocytes. Normal individuals react to this test with a leucocytosis.³

¹ See Schneider (Deutsch. Med. Wchnschr., 1915, XLI, 303) for a discussion of leucopenia following typhoid inoculation. Ziersch, Münch. med. Wchnschr., 1915, LXII, 1310; Austin and Leopold, Jour. A. M. A., 1916, LXVI, 1084; Wells, Jour. Infect. Dis., 1917, XX, 219.

² Camp and Baumgartner (Jour. Exper. Med., 1915, XXII, 174) show that severe inflammatory processes may be associated with a leucopenia.

³ See Mauriac, Jour. de Med., de Bordeaux, 1921, XCII, 373; Sömjen, Med. Klinik, 1921, XVII, 1203; Pagniez and Plichet, Bull. de la Soc. Méd. des Hôp., 1921, XLV, 988.

(F) Variations in Infancy and Childhood.

In the first few days after birth the leucocytes may reach as high as 20,000, while in the nursing period the cells average 13,000. In this connection the influence of a digestion leucocytosis must be remembered as the cells may reach as high as 25,000 of which the lymphocytes constitute about 60 per cent. As the age of the child increases, the number of leucocytes gradually diminishes until the age of puberty, when the average number is about 8,000. As the development of the child progresses, the number of lymphocytes is gradually diminished and that of the neutrophiles correspondingly increased. The relations between the lymphocytes and the neutrophiles must be constantly remembered in making a blood count, as the percentage of lymphocytes is high at first and then gradually diminishes until the age of puberty; the neutrophiles during the same period are at first low, reaching the normal 65 to 70 per cent. about the age of 15. There are no marked differences in the morphology of the granular and of the nongranular cells of the child as compared with those of the adult. The characteristic changes in the leucocytes of the blood of the growing child are more related to the changes consequent upon development and show no signs of degenerative changes.

(G) Functions of the Leucocytes.

The functions of the leucocytes are largely related to their powers of overcoming the effects of bacterial processes. These results are accomplished both through their ameboid powers and their characteristic property of phagocytosis. This latter property is largely influenced by chemotaxis as well as by the indefinite increase of so-called opsonins. Just what we are to regard as the basis of the opsonic index of the leucocyte is unsettled, but it is certain that the same leucocyte may show marked variations in its power of absorbing bacteria of different types. Whether this opsonic power is related in any way to the various assumptions embraced by Ehrlich's side-chain theory must be left for a later section. Besides the above important functions of the leucocytes, these cells show oxidizing, reducing, and fermentative powers, all of which are of more or less importance in the study of immunity and of reaction to bacterial infection. As stated (p. 506), the oxygen catalysts of the blood include oxidase, peroxidase, catalase and hemoglobin. To these ferments we owe many chemical tests for blood and pus, which are destroyed if the specimen be previously boiled, while hemoglobin is still capable of inducing certain oxidations. Kastle and Amoss¹ have studied the peroxidase activity of the blood in health and disease, while Kastle² has made an exhaustive study of the oxidases. Winternitz and Meloy,³ Winternitz and Pratt,⁴ and, especially, Winternitz, Henry and McPhedran⁵ have

¹ Bull. 31, Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Serv., 1906.

² Bull. 59, Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Serv., 1909. See Schultze, Münch. med. Wchnschr., 1909, LVI, 167; Dunn, Jour. Path. and Bacteriol., 1910, XV, 20; and Klopfer, Ztschr. f. exper. Path. u. Therap., 1912, XI, 467; Evans, Arch. Int. Med., 1915, XVI, 1067; Reed, Jour. Biol. Chem., 1916, XXVII, 299; Graham, Jour. Med. Res., 1916, XXXV, 231.

³ Jour. Exper. Med., 1908, X, 759.

⁴ Jour. Exper. Med., 1910, XII, 1 and 115.

⁵ Arch. Int. Med., 1911, VII, 624. See, also, Rohdenburg, New York Med. Jour., 1913, XC VII, 824; Waentig and Gierisch, Fermentforsch., 1914, I, 165; Burge, Jour. Biol. Chem., 1919, XXXVII, 343; Bodansky, Ibid., XL, 127; Reimann and Becker, Am. Jour. Physiol., 1919, L, 54.

turned their attention to catalase. The technic of these latter workers is as follows: 0.025 c.c. of blood is taken from a puncture of the ear by means of a specially graduated pipet. This is immediately diluted with 10 c.c. of distilled water, giving a dilution of 1:400. Five c.c. of this diluted blood are placed in each of two 100 c.c. salt-mouth bottles, one being used for the test, the other being held as a check. In one is placed a small vial containing 5 c.c. of neutralized commercial hydrogen peroxid (3 per cent.). The large bottle is connected with a gas buret, to collect the gas formed by the action of the catalase upon the H_2O_2 . The bottle is agitated for one minute, readings being taken every 15 seconds. Eighty per cent. of the normal cases studied show a liberation of 14 to 17 c.c. of oxygen in 15 seconds, this being constant for the same individual for a period of many months. The importance of this test rests in the variations from day to day or in any change from the normal activity of the individual's blood. The amount of gas liberated is, therefore, a personal rather than a general factor. Tschernoruzki¹ has recently shown that 1 gram of leucocytes is capable of liberating 312.9 c.c. of molecular oxygen under the influence of catalase.

The diagnostic value of this test in disease is as follows: In typhoid fever there is no change in the early stages, but toward the third week there is a gradual fall accompanying the anemia. During the course of lobar pneumonia a slight decline may be noted. Diabetes mellitus shows no change. In diseases of the thyroid, the catalytic activity is not constant from day to day, in hyperthyreosis a tendency to increase being noted while in hypothyreosis a lower level than normal is observed. In renal disease we find no change as long as no marked renal insufficiency obtains, but the activity becomes irregular from day to day, and, as uremia approaches, becomes much lower than normal. With retention of urine due to obstruction of the lower urinary tract a marked decline is observed. Little change is noted in cardiac cases even in the severe types. The toxemias of pregnancy are separable into two classes by this test; without renal involvement eclampsia shows no change but, when renal insufficiency obtains, a marked decline prevails. This test should be of much value in diagnosis.

(7) Blood-plates.

(a) Appearance.

These bodies which have been called "third corpuscles" are probably not true cellular entities. Hayem has considered them as the direct forerunners of the erythrocytes and has styled them, therefore, "hematoblasts." These bodies have been variously called plaques by Osler, blood-plates by Bizzozzero, and by Arnold fragments of cells.² These so-called corpuscles are small colorless bodies containing no hemoglobin. They are about 3 microns in diameter, round, oval, or rod-shaped without any biconcavity. They appear bluish, homogeneous or occasionally granular, and stained lightly by both

¹ Ztschr. f. physiol. Chem., 1911, LXXV, 216.

² See Downey, *Folia Hæmat.*, 1913, XV, 25; Brown, *Jour. Exper. Med.*, 1913, XVIII, 278; Winogradow, *Folia, Hæmat.*, 1914, XVIII, 207; Lee and Minot, *Cleveland Med. Jour.*, 1917, XVI, 65; Wright and Minot, *Jour. Exper. Med.*, 1917, XXVI, 395; Emmel, *Jour. Med. Res.*, 1918, XXXVII, 67. Erede, *Policlinico*, 1921, XXVIII, 203.

basic and acid dyes. They do not contain any nucleus or membrane and become hyaline and glassy as soon as removed from the vessel, but on standing they become pale and unite to form granular masses. They are very sticky and adhere very extensively to one another, forming masses from which fibrinous threads radiate. This fact has led to the belief that they have an important part in the formation of fibrin. (See Duke.¹)

Specimens of the platelets are best obtained by puncturing the tip of the finger or the ear through a 10 per cent. solution of sodium metaphosphate. In this way the blood becomes at once mixed with the fixing fluid and the drop may then be placed on a slide and covered with a cover-glass. It has been customary to call anything a platelet which is smaller than a red-blood cell and which does not contain hemoglobin. The term platelet, however, should be reserved more particularly for these bodies which have a peculiar bluish refractility, no nucleus, show marked cohesive properties, and soon disintegrate.

(b) Size.

The normal size of the blood-platelet averages about 3 microns, although Preisich and Hein have reported them as high as 7 microns. As a rule, their size varies inversely as their number. Some of these bodies show clear areas either in the center or on one side, or on the whole periphery; others become crescents, triangles, spindles and threads (Emerson).

(c) Number.

The normal number of platelets per cmm. is approximately 250,000, this number varying in the same person at different times of day. The physiological factors influencing the number of these cells are not well understood and in pathological conditions we may find large or small numbers of these cells. It is generally accepted that they are increased in anemias from any cause and may be related to the red blood-cells as 1 to 10. They are usually normal in chlorosis, increased in severe secondary anemia and decreased in pernicious anemia. In splenomyelogenous leukemia we find a large increase of these bodies, while in the lymphatic leukemia these cells are diminished. They are increased in chronic diseases associated with cachexia and malnutrition, being more marked in cancer, nephritis and tuberculosis² than in anemia, due to other causes. The more acute, more severe, more threatening the disease, the fewer the number of platelets³ so that we have a direct relationship between severity of disease and number of these bodies. The method of counting these cells has been given in a previous section to which the reader is

¹ Jour. Am. Med. Assn., 1910, LV, 1185; also, Arch. Int. Med., 1912, X, 445.

² See Webb, Gilbert and Havens, Arch. Int. Med., 1914, XIV, 743; Webb and Gilbert, Jour. Am. Med. Assn., 1914, LXIII, 1098.

³ See Duke, Arch. Int. Med., 1913, XI, 100; also, Port and Akiyama, Deutsch. Arch. f. klin. Med., 1912, CVI, 362; Nobécourt and Maillot, Bull. Soc. de Pédiat., 1914, XVI, 285; Weltmann, Wien. klin. Wchnschr., 1914, XXVII, 1013; Dimond, Jour. Path. and Bacteriol., 1915, XIX, 508; Duke, Jour. A. M. A., 1915, LXXV, 1600; Fonio, Cor. Bl. f. Schweiz. Aerzte, 1915, XLV, 1505; Deutsch. Med. Wchnschr., 1916, XLII, 1344; Le Sourd and Pagniez, Ann. de Med., 1916, III, 1; Minot and Lee, Arch. Int. Med., 1916, XVIII, 474; Minot, Ibid., 1917, XIX, 1062; Marchesini, Policlinico, 1920, XXVII, 227; Degkwitz, Ztschr. f. exp. Med., 1920, XI, 144; Deleourt-Bernard, Arch. Med. Belg., 1921, LXXIV, 210; Schilsky, Ztschr. f. klin. Med., 1921, XCI, 256.

referred. Just exactly what significance is to be attached to their increase or decrease is uncertain, but it must be remembered that there is a certain relationship between their number and the severity of general conditions.

(d) Staining Properties.

These bodies stain very much like nuclear material with a basic stain, but also take the acid dyes under certain conditions. With the Wright stain these cells are seen as distinctly blue bodies grouped in numbers from 1 to 10 and seem to be composed of nucleus and protoplasm. The apparent nucleus consist of rows of blue or reddish dots occasionally arranged in spherical masses, while the indefinite poorly-defined protoplasm-like substance seems swollen to almost the size of a red corpuscle. As a rule, however, these bodies appear as grouped bluish masses of indefinite structure and outline.

(e) Function.

The function of these cells is very indefinite. It is possible that they have much to do with the formation of fibrin and may be the source of the so-called fibrin ferment, thrombogen.¹ If we are to regard these bodies as derived from the leucocytes this function of fibrin formation is acceptable, but if they are to be considered as derivatives of red cells or as true independent bodies such an hypothesis is untenable. No facts of any clinical value have so far been forthcoming from the study of the blood plates, and it may be possible that they are really artefacts as Löwit has claimed.

(8) Hemoconien.

By this term we have reference to the presence in normal blood of very fine granules which are actively motile, not truly ameboid, but with motion more of the Brownian type. These granules, also called blood-dust by Müller, are small, round, colorless granules which vary in size from very fine dust-like particles to some as large as 1 micron in diameter. Their exact chemical nature is uncertain as they do not show, according to Müller, either the reactions of fat or of albumin. The general idea prevails that these bodies are the extruded granules of the leucocytes, as they resemble in size and in staining qualities those of various leucocytic types. The number of these granules is uncertain, but apparently the relation of these granules to the red cells, as shown by the ultra condenser, is about 50 to 1.

Although no clinical significance is known to attach to these granules, the writer has observed marked variations in their number in various pathological conditions, but cannot at present draw any conclusions from such observations.

(9) Morphology of the Blood-forming Organs.

As the bone-marrow is of such importance in the production of both red and white cells, it would seem advisable briefly to discuss the histology of this tissue. In it we find practically every cell which occurs in the blood both in normal and abnormal conditions, and also many transitional forms between

¹ Bayne-Jones (Am. Jour. Physiol., 1912, XXX, 74) has shown that the platelets aid in the clotting of blood in two ways: (1) by setting free prothrombin, which is later activated to thrombin, and (2) by liberating a thromboplastic substance which neutralizes the anti-thrombin normally present in the blood.

various groups of cells. The following brief outline of the histology of the bone-marrow is taken from a valuable paper of Dickson.¹

Varieties of Bone-marrow.

(1) Primitive or Embryonic Marrow.

A delicate interlacing network of mucoid cells, which later in the process of development go to form the connective-tissue framework or adenoid reticulum of the tissue.

(2) Red "Lymphoid" or Formative Bone-marrow Proper.

This is found in the adult in the short and flat bones, sternum, ribs and vertebræ, and to a varying extent in the ends of the long bones. This is the most important variety as in it are found the red and the majority of the white cells of the blood, and, according as one or the other of these series of cells predominates, the type of marrow may be classified as erythroblastic or leucoblastic, a varying admixture of these two types being practically always found in any given case.

(3) Fatty or Yellow Marrow.

This is found mostly, as age advances, in the central part of the long bones and is formed by a process of physiological transformation or degradation of the connective-tissue elements, together with the gradual disappearance of most of the blood-forming cells of the red marrow.

(4) Fibroid Marrow.

This is found in old persons, especially if debilitated by long-standing disease, and is characterized by the proliferation of the connective-tissue elements and by the progressive sclerosis of the marrow, followed by the gradual disappearance of the hemopoietic cells of the tissue.

(5) Gelatinous Marrow.

This is essentially a retrogressive change in the tissue and is in no way identical with that type already described as primitive or embryonic marrow. This change has been described by a previous writer as a chronic condition only, but has been frequently found by Dickson as an acute change in many of the acute infectious fevers and allied diseases.

Cytology of the Bone-marrow.

In this discussion little more will be taken up than a brief enumeration of the various varieties of cells, as the more important ones of these have been treated in other sections.

(I) Blood-forming Cells.

(A) Leucocyte Series.

(a) Nongranular Cells with Basophile Protoplasm.

(1) Large.

(2) Small.

(α) Cells similar to but smaller than the large variety.

(β) Cells identical in appearance and in staining reactions with the small lymphocyte of the blood. Of these there are also probably two varieties.

¹ Jour. Path. and Bacteriol., 1907, XII, 136.

(b) Granular Cells.**(1) Neutrophile.**

- (α) Myelocytes with large rounded or oval nuclei. These have been definitely traced by Dickson to nongranular or hyaline cells in which the granules may be seen gradually developing. There are two types known as the larger and the smaller neutrophile myelocyte.
- (β) Intermediate cells with indented or horse-shoe-shaped nuclei. These are developed from the myelocytes (α) and in turn go to form
- (γ) Polymorphonuclear cells or adult leucocytes which pass out into the blood stream.

(2) Eosinophile.

The same types of these cells with eosinophile granulations are found in the marrow, as have been previously tabulated under the heading of neutrophile cells.

(3) Basophile.**(α) Mast cells.**

The three types above discussed are also observed in the basophile mast cells of the bone-marrow.

- (β) Cells resembling the eosinophile myelocytes but with granulations staining with the basic dye. These cells are probably altered eosinophiles.

(B) Hemoglobin-holding Series.

- (1) Normoblasts. Normocytes or ordinary red corpuscles.
- (2) Megaloblasts { Normoblasts, normocytes.
 { Megalocytes (entirely pathological in the adult).

(II) Giant Cells.

- (1) Mononucleated or megakaryocytes.
- (2) Multinucleated or polykaryocytes.

(III) Cells of Connective-tissue Type.

- (1) Fat cells.
- (2) Cells of the reticulum.
- (3) Various forms of phagocytic cells.
- (4) Ordinary connective-tissue cells.

(IV) Endothelial Cells.

- (1) Found in their normal position in the vessel wall.
- (2) Found proliferating and taking on phagocytic functions.

Reactions of the Bone-marrow in Disease.

Many of these reactions are connected with the production of the so-called inflammatory leucocytosis and take place with great rapidity. Other varieties of change are intimately concerned with the production of the hemoglobin-holding series of cells. These reactions may, according to the type of cell involved, be summarized as follows:

(I) Leucoblastic.

(1) Neutrophile.

(3) Basophile.

(2) Eosinophile.

(4) Hyaline or non-granular.

(II) Erythroblastic.

(1) Normoblastic.

(2) Megaloblastic.

The above brief outline will show the reader that we have in the marrow all possible types both of red and of white cells and that any variation in the normal activity of the marrow will result in the overloading of the blood with cells of a particular type depending upon the kind and extent of the affection.

The histology of the other blood-forming organs, as the liver, spleen, and hemolymph nodes may be found in any text-book on histology.

IV. PATHOLOGY OF THE BLOOD.**(I) Special Pathology.**

Under the head of the special pathology of the blood we have to consider the conditions which are manifested directly by changes in the composition and cellular structure of this tissue. In very few of the blood diseases proper is the blood picture so characteristic that a definite diagnosis is always possible, but in a few of them certain changes are more frequently found and more often lead to a presumptive diagnosis. The pathological conditions in the blood may be considered either primary or secondary, but it should be remembered that severe secondary changes may so closely simulate those found in primary conditions that differentiation is almost impossible. It is probably true that all pathological changes of the blood are really secondary, but in a certain number of these states the etiologic factors are so obscure that we can do no more than interpret the blood findings as primary conditions.

(A) Anemia.

This is a condition characterized by a deterioration, both qualitatively and quantitatively, in one or in all of the blood constituents. It is usually characterized by a diminution in the percentage of hemoglobin (oligochromemia) and by a decrease in the number of red cells (oligocythemia), but we should regard as essential factors in the anemic condition a reduction in the total volume of blood (oligemia) as well as a reduction in the amount of protein (hypalbuminosis). As much more attention has been paid to the first two factors than to the latter ones, anemia has come to mean a reduction in the amount of hemoglobin with a more or less extensive reduction in the number of red cells. Associated with these conditions we have, in the severer types of anemia, variations in form, size, and structure of the red cells as well as definite changes in the relationship of the different white cells. In the study of the anemic conditions we must differentiate the primary from the secondary form, by this we mean a differentiation between those forms which have no demonstrable cause from those types whose etiology is more or less secondary to other pathologic conditions.

Primary Anemias.**(1) Simple Primary Anemia.**

This form which has no demonstrable cause is difficultly separable from

the secondary form as well as from certain other primary forms, such as primary pernicious anemia. It will probably be shown to be a true secondary anemia and must be sharply differentiated from the pernicious type from the point of prognosis. These cases are only recognizable when they are typical in form and are frequently not amenable to any form of treatment which we may institute. The rule, however, is that these forms of anemia yield more or less promptly to proper dietetic and therapeutic treatment. It is probable that the question of prognosis in these cases must depend largely upon the amount of degeneration of the red cells which occurs.¹ In these cases we find that the diminution in the number of red cells is usually parallel to the reduction in the amount of hemoglobin, so that a high color index will usually obtain. More or less degeneration, as evidenced by the appearance of poikilocytes, normoblasts, Maragliano's polychromatophilia, etc., will be observed depending upon the severity of the case. The leucocytes are usually normal in number and in differential relations, while the blood plates are usually increased. The changes in the plasma in this primary anemia are not characteristic, although we do observe a diminution in the specific gravity which runs parallel to the oligochromemia.

(2) Chlorosis.

Chlorosis is a primary anemia occurring almost exclusively in girls about the age of puberty and characterized by a marked reduction in the amount of hemoglobin and a slight change in the number of red cells. Clinically, this state is evidenced by the appearance of a wax-like changing into a greenish tone of the skin and a sky-blue coloration of the cornea. Some cases may show a variety of colors of the skin. Certain changes are observed in the digestive and generative organs and certain general abnormalities are seen which are due to lessened production of blood-cells and to diminished oxidative and fermentative powers of the system. This form of primary anemia differs from all other forms in the absence of blood degeneration, as very rarely marked degenerative signs appear in the blood picture. The blood finding is not absolutely characteristic for this clinical entity, as it is simulated by many anemias of the secondary type. Clinically, this disease is so sharp that a diagnosis is often possible without a blood examination.

The chief characteristics of the blood in this condition are: (1) Reduction in the hemoglobin. This may run as low as 20 per cent., giving a color index of 0.5. Secondary anemias rarely reach such a low level. (2) Variations in the number and size of the red cells. The number of red cells is not reduced to a very great extent, the average being about 4,000,000, although counts as low as 1,000,000 have been reported. When these low counts do occur some complication should be suspected. Ordinarily the size of the cell is diminished, although we frequently find large "dropsical" cells which are due to absorption of fluid from the hypotonic plasma. These latter cells are usually few in number, the great majority of cells being smaller than the normal size.

¹ See Pollitzer (*Ztschr. f. klin. Med.*, 1912, LXXV, 367) for a discussion of the types of regeneration and degeneration in anemia; also, Wichern and Piotrowski, *Deutsch. Arch. f. klin. Med.*, 1912, CVI, 533; Pribram, *Deutsch. Arch. f. klin. Med.*, 1914, CXVI, 535; Kleinschmidt, *Jahrb. f. kinderhkd.*, 1916, LXXXIII, 97.

Poikilocytes and degenerated reds rarely occur except in the severer forms of this disease, while chromatophilia is usually regarded as a sign of active regeneration of the blood. When nucleated reds occur, which is a rare finding, they are practically always of the normoblastic type and rarely appear of the megaloblastic form.

The leucocytes in this condition are usually normal both in size and number and degenerative forms are rarely seen. It has been stated that the eosinophile cells are much increased in this condition, but the writer has found that their ratio is very rarely above the upper limit of the normal figure for these cells. The platelets are usually about normal and are usually large in size.

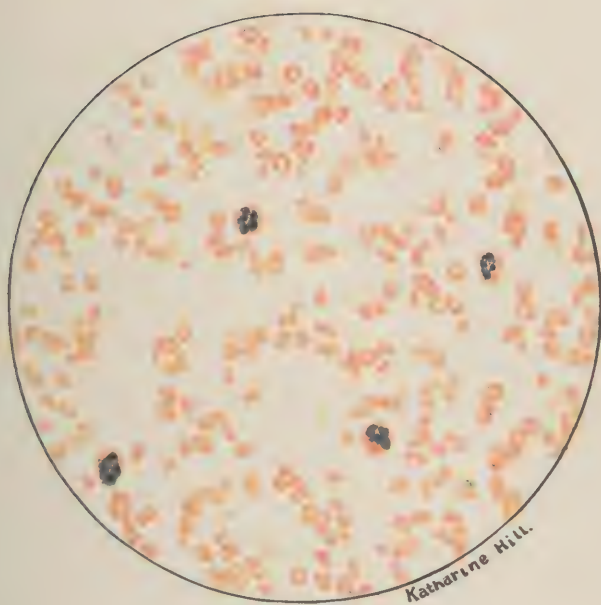
In chlorosis we find certain variations in the physical and chemical properties of the blood. The specific gravity is usually reduced in proportion to the reduction of the hemoglobin and may fall as low as 1028. The plasma is diluted so that a condition of oligemia may be considered more or less characteristic of chlorosis. Whether or not a hydremia, as indirectly manifested by a diminution in the amount of albumin, obtains, is debatable.¹

Chlorosis belongs to the class of primary anemias and as such has no definite etiology. Various conditions such as hypoplasia of the arterial system, intestinal autointoxication, disturbances of the nervous system, such as a vasomotor neurosis, have been advanced to explain this condition, but none of them are tenable in all cases. A great importance must attach to hygienic conditions, poor food, and mental depression, especially about the age of puberty, as this disease is usually apparent under these conditions at this time of life. A further factor which must necessarily bear upon the etiology of this condition is the defective power of absorption of iron compounds. Patients afflicted with chlorosis improve rapidly under the administration of iron, but not unless the digestive and absorptive powers improve at the same time. It does not concern us here as to the dynamics of the absorption and the effect of iron, but it should be accepted as an axiom that no therapeutic effects may be expected from iron unless the absorptive power is made better. Just exactly what the pathological conditions are which are accountable for the functional insufficiency of the bone-marrow is uncertain, but it must be recalled that gross as well as microscopic pathologic changes are not necessary to produce functional disturbance in any organ.

It should be remembered that we may have various types of chlorosis which show different prognostic characteristics: (1) Those in which the red cells are very slightly reduced (about 4,000,000), a marked diminution in the amount of hemoglobin, a low color index, and no change in the size and shape of the cell. Such cases usually recover promptly without showing any relapse. (2) Cases in which the red cells are below 4,000,000, which show a marked diminution in the amount of hemoglobin with a very low color index and which give very slight evidences of degenerative changes in the red cells. These cases are usually characterized by marked prostration, but usually recover more or less promptly although relapses frequently occur. (3) Cases

¹ See Frohmaier, *Folia Hæm.*, 1915, XX, 115.

PLATE XXIII.



CHLOROTIC ANEMIA. (WRIGHT'S STAIN.)

in which the red cells are reduced as low as 2,500,000, a reduction in the hemoglobin with a very low color index, marked changes in the shape and size of the red cells. These cases respond slowly to treatment and have a bad prognosis (Ewing).

As these cases of chlorosis convalesce, we observe an increase in the number of red cells to a point somewhat above the normal and a later increase in the hemoglobin content of each individual cell. These changes are usually evident in from eight to ten days after institution of treatment, but as a rule a much longer time is necessary for any marked change to be observed in the number of the cells or in their hemoglobin content. The changes in the plasma are usually the first to appear and should be considered essential for the proper regeneration of the blood in chlorotic conditions.

This disease belongs in the group of curable conditions and usually has a good prognosis, but the susceptibility to intercurrent acute diseases during this period is much increased so that we should always bear in mind the probability of complications arising in the convalescent period.

(3) **Progressive Pernicious Anemia (Biermer's Anemia).**

This term is applied to a form of severe anemia which, in spite of all treatment, progresses steadily toward death. It results from defective hematogenesis and excessive hemolysis and is characterized by definite changes, both numerically and morphologically, in the red cells and by characteristic changes in the bone-marrow (Ewing). This condition has frequently been described as the result of infection with certain intestinal parasites so that it cannot in all cases be considered a truly primary disease, although the larger majority of cases show no known etiology.¹ According to Herter, certain anerobic bacteria, found in the large intestine, produce a substance of marked hemolytic power, which penetrates the intestinal wall and enters the portal circulation.² In some cases of idiopathic purpura hemorrhagica the blood picture is that of pernicious anemia, but may be distinguished by the absence of megaloblastic change and by the prominence of hemorrhage.³ The blood picture in this disease is not absolutely characteristic, as certain forms of secondary anemia may show similar findings. The chief characteristics of the blood in primary pernicious anemia, as also in those severe types of secondary anemia which simulate this form, are (1) signs of rapid blood destruction, such as degenerated reds, endoglobular degenerations, polychromatophilia, increased iron compounds in the serum and corpuscles, an increase of iron in the liver and spleen;⁴ (2) poikilocytosis; (3) a high color index resulting from a marked diminution in the number of red cells and a correspondingly less degree of diminution in the amount of hemoglobin; (4) megaloblastic blood formation. This latter indicates a direct reversion to the embryonal

¹ See Friedstein, Inaug. Dissert., Berlin, 1912; Lüdke and Fejes, *Deutsch. Arch. f. klin. Med.*, 1913, CIX, 433; also Pilcher, *Am. Jour. Med. Sc.*, 1913, CXLVI, 226; Ragosa, *Folia Hæmat.*, 1915, XIX, 269.

² See Banti, *Semaine méd.*, 1913, XXXIII, 313; also, Robertson, *Jour. Biol. Chem.*, 1915, XVI, 652.

³ See Minot, *Am. Jour. Med. Sc.*, 1916, CLII, 48.

⁴ McMaster, Rous and Larimore (*Jour. Exp. Med.*, 1922, XXXV, 521) have shown that this hemosiderosis is not especially significant of pernicious anemia.

type of blood formation in which the presence of megaloblasts as direct precursors of megalocytes is observed.¹ The red blood-cells in this condition are few in number being reduced to as low as 1,000,000 cells, counts of 500,000 having been observed without the patient suffering any marked inconvenience. Naegeli reports a case showing 138,000 reds. This fact should be taken as evidence that the oligocythemia in pernicious anemia is not alone accountable for the symptomatology. This count may remain stationary, may show slight decrease, but usually progresses slowly until death ensues. The average diameter of the red cells is somewhat increased in pernicious anemia. While many of them may be normal in size and many very small, the cells average from 4 to 13 microns in diameter. A pernicious anemia is a distinct large cell anemia. A macrocytosis is much more characteristic of this disease than of any other, 70 per cent. of the cells in these cases being of this type.² Microcytosis is rare but may occur to such an extent that the average size of the red cell may be about normal. Poikilocytes are very common and often show extreme shapes and are frequently numerous in number. Polychromatophilic degeneration is very extensive in this form of anemia.

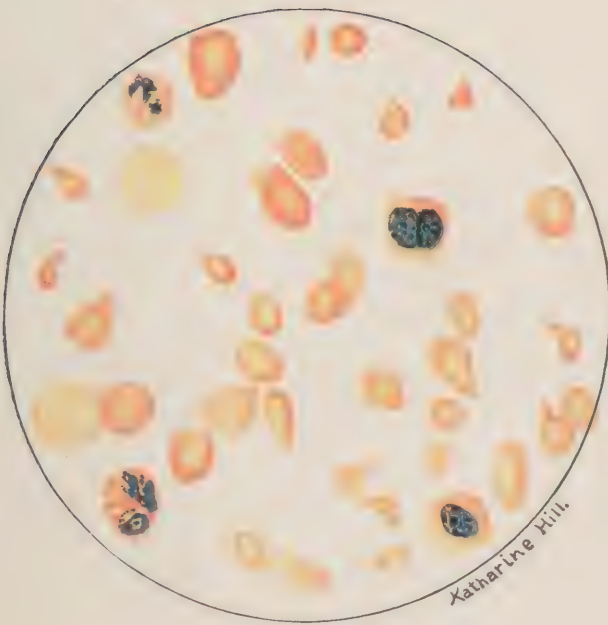
While both normoblasts and megaloblasts occur in large numbers in pernicious anemia, the megaloblasts usually outnumber the normoblasts. This megaloblastic increase may be considered pathognomonic of this anemia, as a preponderance of these large nucleated red cells does not occur, except in rare cases, in the other varieties of anemia. The hemoglobin may be markedly reduced showing values rarely above 50 per cent. and often as low as 10 per cent. The color index is always high; being more frequently above one than below. A low index is found, according to Ewing, in the chronic cases, while the acute forms are more frequently associated with a high index. If improvement occurs the index is always lowered, an increasing index denoting a bad prognosis.

In this pernicious form of anemia we find the leucocytes practically always diminished in number, averaging about 4,000, a condition which almost never obtains in a secondary anemia. Their number usually runs parallel to that of the red cells, a leucocytosis pointing probably to a complication. As the case improves the neutrophile cells increase in number, the low leucocyte count being, as a rule, due to their diminution. The percentage of the non-granular mononuclear cells varies inversely to that of the granular form. As the disease progresses the percentage of the nongranular cells increases, while the granular cells are diminished. This disease shows, therefore, a high lymphocyte count which is relative and not real, being due to the diminution in number of the polymorphonuclear cells. The lymphocytes may constitute as high as 50 per cent. of the leucocytes, the eosinophiles may reach as high as 10 per cent., the myelocytes 2 per cent., while the mast cells may run as high as 3 per cent. Degenerations of all kinds are observed in the leucocytes,

¹ See Brösamlen, *Deutsch. Arch. f. klin. Med.*, 1913, CXII, 83; Vogel and McCurdy *Arch. Int. Med.*, 1913, XII, 707; Roth, *Ztschr. f. klin. Med.*, 1914, LXXIX, 266.

² See Dünner, *Berl. klin. Wchnschr.*, 1914, LI, 1759.

PLATE XXIV.



BLOOD IN PERNICIOUS ANEMIA. (WRIGHT'S STAIN.)

but nothing characteristic of pernicious anemia is found in the white cells.¹

The blood-platelets are largely decreased and may be as low as one-twenty-fifth of the normal number. Von Limbeck and Sahli claim that these cells are increased in number, but the usual finding is one of diminution.

(4) Splenic Anemia.

This is a form of chronic anemia characterized by idiopathic enlargement of the spleen without any involvement of the lymph nodes. A large number of conditions may be responsible for the anemia of the splenic type, so that a direct etiologic factor should be looked for in all cases. Among the conditions which may give rise to this type of anemia, we find gummata of the spleen, large round-cell sarcoma of the spleen, chronic splenitis of the malarial type, and splenomegaly associated with cirrhosis of the liver. This latter condition is known as Banti's disease and its etiology is uncertain.²

While it is true that the splenic lesions do not differ essentially from certain stages of the lesions in ordinary pseudoleukemia, yet we do not find in this condition any involvement of the lymph-glands, although the spleen may be enormously enlarged. The blood picture in this condition is characterized by a relatively high red cell count, a marked reduction in the percentage of hemoglobin, and a consequent low color index. The white cells are rarely increased, a leucopenia being rather the rule. When we do find an increase in the number of white cells a relative lymphocytosis occurs usually associated with an increase in the number of basophiles. Poikilocytes and nucleated red cells are very uncommon while degeneration may occasionally be observed. Whether or not we have an increase or a decrease in the number of leucocytes, a relative lymphocytosis associated with enlarged spleen and no involvement of the lymph nodes must be considered characteristic of splenic anemia.

In Banti's disease we find an enormous increase in the size of the spleen and associated with this an extensive cirrhosis of the liver. Along with these factors we find a high-grade toxogenic protein decomposition, which is

¹ See Nicol, *Deutsch. Arch. f. klin. Med.*, 1913, CXI, 417; Sisto, *Policlinico*, 1913, XX, 509; *Ibid.*, 1914, XXI, 34, 91 and 115; Wolff, *Deutsch. Med. Wchnschr.*, 1914, XL, 643; Cederberg, *Berl. klin. Wchnschr.*, 1914, LI, 585; Mikhailoff, *Russk. Vrach.*, 1914, XIII, 837; Cavaglieri, *Gazz. d. osp.*, 1914, XXXV, 1169; Schmidt, *Am. Jour. Med. Sc.*, 1914, CXLVIII, 313; Briggs, *Ibid.*, 413; Moffitt, *Ibid.*, 817; Krumbhaar, *Ibid.*, 1915, CL, 227; Drinker and Hurwitz, *Arch. Int. Med.*, 1915, XV, 733; Vogel, *Jour. A. M. A.*, 1916, LXVI, 1012; Schneider, *Arch. Int. Med.*, 1916, XVII, 32; Roccavilla, *Policlinico*, 1916, XXIII, 281, 329, 367 and 399; Esch, *Ztschr. f. Geburtsh. u. Gyn.*, 1916, LXXIX, 1; Jessen and Unverricht, *Munch. Med. Wchnschr.*, 1916, LXIII, 1787; Gilfin, *Jour. A. M. A.*, 1917, LXVIII, 429; Squier, *Jour. Lab. and Clin. Med.*, 1917, II, 552; Barsky and Kahn, *Proc. Soc. Exp. Biol. and Med.*, 1918, XVI, 31; Kahn and Barsky, *Arch. Int. Med.*, 1919, XXIII, 334; Rombach, *Nederl. Tijdschr. v. Geneesk.*, 1919, II, 671; Marcora, *Policlinico*, 1919, XXVI, 424; Maynard and Sturton, *Brit. Med. Jour.*, 1921, II, 685; Condat, *Arch. de Med. des Enfants*, 1921, XXIV, 676; Benoit, *Bull. de la Soc. Méd. des Hôp.*, 1921, XLV, 741; Ellermann, *Hospitalstid.*, 1922, LXV, 65; Alder, *Schweiz. med. Wchnschr.*, 1922, LII, 172; Mayo, *Ill. Med. Jour.*, 1922, XLI, 175.

² See Banti, *Berlin. klin. Wchnschr.*, 1911, XLVIII, 2328; Stein, *Am. Jour. Med. Sc.*, 1912, CXLIV, 856; also, Ueber, *Munch. med. Wchnschr.*, 1912, LIX, 1478; Lacouture, Dupré, and Charbonnel, *Jour. de méd. de Bordeaux*, 1913, LXXXIV, 805; Caronia, *Pediatrics*, 1914, XXII, 752; Sailer, *Penn. Med. Jour.*, 1914, XVIII, 91; Krull, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1914, XXVIII, 718; Mitamura, *Mitt. a. d. Med. Fak. d. Univ. Tokyo*, 1919, XXI, 245; Garin, *Riv. Crit. de Clin. Med.*, 1921, XXII, 1 and 13.

associated with very high values for the total nitrogen of the urine and of the output of purin bases. The number of erythrocytes diminishes corresponding to the degree of anemia, while the hemoglobin percentage is more markedly reduced. The leucocytes are either normal or more frequently diminished in number, a relative lymphocytosis existing as in the pure type of splenic anemia.¹

(5) **Anemia Infantum Pseudoleukemica.**

Von Jaksch has described a rare form of anemia seen in children which is characterized by enlargement of the spleen, liver, and lymph nodes. The most striking points in this condition are the great diminution in the number of red cells, one case showing only 820,000; numerous nucleated red cells; diminution of hemoglobin; the leucocytes always increased in number, being from 20,000 to 50,000, as a rule, and displaying a remarkable variety of form and frequently attaining to unusual size. The morphological changes in the blood resemble both those seen in leukemia and in pernicious anemia, the disease passing either into one or the other of the previously mentioned conditions. The blood findings are not alone sufficient to warrant a diagnosis of infantile pseudoleukemia, but are significant when taken in conjunction with the clinical findings.²

(6) **Leukanemia.**

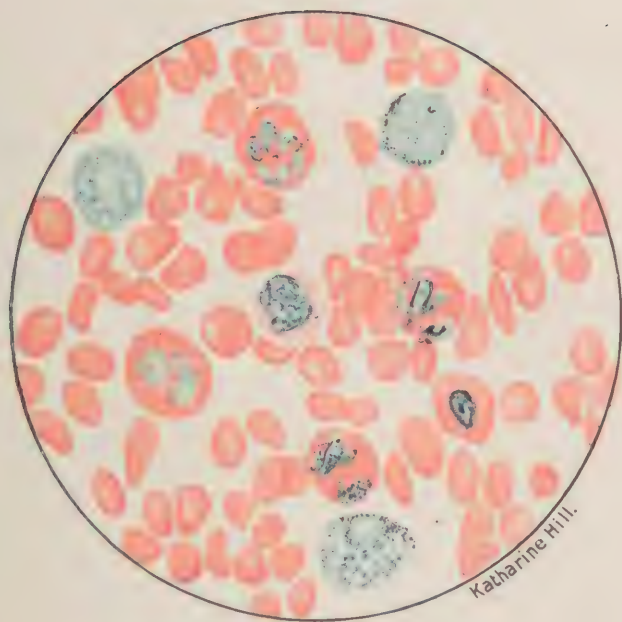
This condition has been classed by some with the leukemias as the blood findings are occasionally more prominent among the leucocytes, while by others it is classed with pernicious anemia owing to the frequent changes in the red cells. Von Leube considers this anemia a mixed form of pernicious anemia and of leukemia. Luce³ regards this as a symptom of many conditions rather than an independent blood disease. In the majority of these cases the changes are more evident in the red cells, marked diminution in number (as low as 200,000 per cmm.) associated with extensive destruction of red cells with all irregular and unusual types of these cells being observed. The diminution in the amount of hemoglobin is great, but the color index is usually high just as we find it in pernicious anemia. The white cells show an extensive disturbance in the neutrophile and eosinophile blood picture with a large increase in the number of large lymphocytes. The number of white cells is usually increased, but not to as great an extent as in leukemia. The changes in the red cells and in the hemoglobin usually precede those in the white cells, so that the early stages may show us a typical picture of pernicious

¹ Yates, Bunting and Kristjanson (Jour. Am. Med. Assn., 1914, LXIII, 2225) report the isolation of pure cultures of *Bacillus hodgkini* (see Chap. XI) in two cases of Banti's disease. They believe there is an etiologic relationship here. See, also, Gibbons (Quart. Jour. Med., 1914, VII, 153), who reports the presence of a streptothrix in six cases. Paiseau and Lemaire, Arch. des Mal. du Cœur, 1916, IX, 473; Humphrey, Allbutt, Deighton and Hare, Brit. Med. Jour., 1916, I, 365; Eberly, Jour. A. M. A., 1916, LXVII, 33; Joseph, Ibid., 1936; Norris, Symmers and Shapiro, Am. Jour. Med. Sc., 1917, CLIV, 803.

² See Stillman, Am. Jour. Med. Sc., 1917, CLIII, 218; Kilduffe, Arch. Diag., 1917, X, 6; Evans and Happ, Bull. Johns Hopk. Hosp., 1922, XXXIII, 1.

³ Deutsch. Archiv. f. klin. Med., 1900, LXXVII, 215. See, also, Martelli, Virchow's Arch., 1914, CCXVI, 224; Gazz. internat. di med., 1914, XVII, 553 and 577. Allan and Leinbach, Jour. A. M. A., 1917, LXVIII, 1020; Wanner, Schweiz. Med. Wehnschr., 1920, L, 685; Symmers, Jour. Am. Med. Assoc., 1921, LXXVI, 156.

PLATE XXV.



Katharine Hill.

BLOOD IN LEUKANEMIA. (WRIGHT'S STAIN.)

anemia, while later examinations may lead to the diagnosis of the mixed condition.

(7) Aplastic Anemia.

Ehrlich has reported a rapidly progressing anemia accompanied by hemorrhages into the mucous membranes, associated with hyperplasia of the bone-marrow, and not showing the ordinary changes in the blood which are supposed to accompany pernicious anemia. This type has been called aplastic anemia and has not been frequently reported.

The red cells are usually markedly reduced in number, being as low as 790,000 in a case reported by Wood. The hemoglobin may fall as low as 11 per cent. as reported by Muir, while the leucocytes are usually normal in number but showing much reduced percentages of the polynuclear neutrophils. In this condition we find an enormous increase in the number of lymphocytes, the percentage being in Lipowski's case 93, the remaining 7 per cent. being neutrophils. No nucleated reds have been found in the blood and only a very few in the marrow itself, which is fatty, almost white and contains few neutrophils and no eosinophils.¹

Secondary Anemia.

By secondary anemia we mean one in which etiological factors seem sufficient to explain the variations observed in the blood. The principal variation seems to be more directly observed in the reduction of the hemoglobin, although the number of red cells is coincidentally reduced, but not to the same degree as is the hemoglobin. In the mild cases the color of the blood is but slightly paler than normal, but in secondary anemia of a severe type the color may resemble the watery drop observed in cases of typical pernicious anemia.

Cabot suggests a classification of the secondary anemias as follows: (1) Mild cases, showing a normal count of red cells, but having the hemoglobin diminished. (2) Moderate cases in which the count is normal but the cells show signs of moderate degeneration, abnormal staining qualities, and a diminished tendency to rouleaux formation. (3) Severe cases, in which the count is not much reduced but in which the hemoglobin is very much lessened and the cells show marked qualitative and quantitative changes. (4) Very severe cases with a slightly lessened blood count, a marked diminution of the hemoglobin, and evidences of degeneration and destruction of the cells as well as evidence of regeneration. In some of the severer types of secondary anemia the blood picture may so closely resemble that of pernicious anemia that a diagnosis is possible only through the careful investigation of the etiology of the condition.

¹See Hirschfeld, *Folia Haemat.*, 1911, XII, 347; Musser, *Arch. Int. Med.*, 1914, XIV, 275; Kleinschmidt, *Jarb. f. Kinderhkde.*, 1915, LXXXI, 1; Predtechensky, *Russk. Vrach.*, 1915, XV, 313; Frank, *Berl. klin. Wchnschr.*, 1915, LII, 961 and 1062; O'Malley and Conrad, *Jour. A. M. A.*, 1919, LXXIII, 1761. Larrabee, *Jour. Am. Med. Assoc.*, 1920, LXXV, 1632; Larkins, *Arch. Radiol. and Electrotherapy*, 1921, XXV, 380; Gorke, *Deutsch. Arch. f. klin. Med.*, 1921, CXXXVI, 143; Williams, *Lancet*, 1921, II, 74; Spak, *Acta Pediat.*, 1921, I, 370; Herrman, *Am. Jour. Dis. Child.*, 1922, XXIII, 484.

Blood Picture.

The general points observed in secondary anemia are as follows: A variable decrease in the amount of hemoglobin, a reduction in the number of red cells less in degree than the diminution of hemoglobin and a subnormal color index, which rarely reaches the low grade shown in chlorosis. The lowest color index is seen in those secondary anemias following cancer, severe hemorrhage, and gangrenous processes. The specific gravity of the blood is reduced corresponding to the degree of reduction of hemoglobin. The rapidity of coagulation is increased depending upon the grade of oligochromemia and of oligocythemia. The reduction in the number of red cells may be very marked as, for instance, in a case of von Limbeck the number was 306,000. The red cells show a lack of hemoglobin, frequently appearing as the pessary forms. Polychromatophilia is quite common, but bears no direct relation to the hemoglobin content of the cell. Only in the severer cases do we find poikilocytes, although anisocytes, especially microcytes, are frequently present, the larger "dropsical" cells being less common than in chlorosis. Nucleated erythrocytes are frequently seen in some cases, while in others even of severer grade of anemia they are absent. These nucleated reds are, as a rule, of the normoblastic variety, megaloblasts being exceedingly rare.

The leucocytes vary in number depending on the cause of the anemia, from a leucopenia, which is rare, to a leukemic condition. The increase in the number of leucocytes is more frequently in the polynuclear neutrophiles, the lymphocytes being rarely if ever increased, while the eosinophiles, although not increased, are usually at the upper limit of the normal value for these cells. In some cases of the severe chronic types of secondary anemia we may find a lymphocytosis, but this is rare. The blood-platelets are usually increased in number, in some cases being two and one-half times the normal values.

(1) Acute Hemorrhage.

The character of the anemia following an acute hemorrhage will depend upon the type of the hemorrhage, that is whether the loss of blood occurred suddenly and at one period, or whether slowly and at intervals.¹ The loss of one-half of the total volume of blood at one time is usually fatal, as Panum has shown. If the loss be less following one large hemorrhage, regeneration takes place in from five to thirty days, depending upon the amount of blood lost. Regeneration is quickest in men between the ages of 20 and 40, slower in women, and slowest in children.²

Immediately following a hemorrhage the blood picture will be normal qualitatively as there has been no time for the morphologic changes to take place. Shortly following the hemorrhage lymph pours into the blood to restore the volume and maintain the pressure, so that the blood count and the hemoglobin diminish usually to the same degree. As the formation of new cells goes on the color index decreases, as the new cells are, as a rule, deficient

¹ See Milne, *Jour. Exper. Med.*, 1912, XVI, 325; *Deutsch. Arch. f. klin. Med.*, 1913, CIX, 401.

² See Whipple, Hooper and Robschit, *Am. Jour. Physiol.*, 1920, LIII, 151, 167, 206, 236 and 263; Whipple and Robschit, *Arch. Int. Med.*, 1921, XXVII, 501; Musser, *Ibid.*, XXVIII, 638; McMaster and Haessler, *Jour. Exp. Med.*, 1921, XXXIV, 570.

in hemoglobin. These cells are more or less easily degenerated, showing variation both in their shape and staining qualities. The number of cells reaches the normal much sooner than does the percentage of hemoglobin, so that we may find for weeks evidences of marked anemia.

The most frequent causes of acute hemorrhagic anemia are traumatism, ectopic pregnancy, abortion, gastric and duodenal ulcers, uterine tumors, pulmonary tuberculosis, and hemorrhagic pancreatitis.

(2) **Chronic Hemorrhage.**

By a chronic hemorrhage we mean one in which repeated hemorrhages follow one another so closely that the blood has no time to regenerate before a second loss of blood occurs. This rules out of consideration those cases in which repeated hemorrhages occur but at sufficiently long intervals to permit of regeneration of the blood. This latter condition, although a chronic hemorrhage, gives the picture described under Acute Hemorrhage.

In the chronic hemorrhage we find the red cells markedly reduced, the hemoglobin very much diminished and usually a marked leucocytosis. The red cells are usually small and pale, show a low color index, and usually few nucleated forms, although the picture may rarely assume the pernicious type. Regeneration in this form of anemia is slow, as the blood-forming organs seem to lose their power of regenerating the blood after repeated hemorrhages.

The most frequent causes of chronic hemorrhage are scurvy, epistaxis, hemorrhoids, intestinal ulcers, gastric and other carcinomata, and intestinal parasites.

(3) **Inanition.**

In the discussion of the anemia of inanition, it must be remembered that other factors than starvation are necessary in its causation. That starvation alone will not cause anemia is shown from the examination of the blood of Cetti who fasted ten days. His blood showed about 6,000,000 red cells, a small diminution in the percentage of hemoglobin, and a leucocyte count of 4,200. Although the changes in the cells and the pigment of the blood are not marked following starvation, it is true that we have a loss of albumin of the plasma and a diminution in the total volume of blood. This is taken as evidence by Grawitz that a true anemia occurs. Such changes are not striking if the days of fasting are alternated with days of slight nourishment. It is not so much the quantity of food as the quality which is of importance in bringing about the anemic conditions. As is well known, the foods containing iron are the principal sources of the hemoglobin of the blood, and these are frequently, owing to disturbed gastric and intestinal functions, poorly digested and assimilated. Although the amount of iron contained in the ordinary food is sufficient under the best conditions to maintain the hemoglobin content of the blood, yet the methods of food preparation, as well as the abnormal methods of rapid eating, are important factors in the poor assimilation of the iron of the food.

The lack of sunlight as well as impure air are contributory factors in causing anemia through their influence upon the general body functions. No

tissue, whether animal or plant, can flourish in air which does not have sufficient oxygen to support the combustion processes of the system. This statement needs no retraction in the case of anaerobic bacteria, as it has been definitely shown that these organisms obtain the oxygen necessary for their development from the culture media upon which they grow, although they are incapable of developing in an atmosphere of pure oxygen. Overwork, especially when associated with worry, has great influence upon all of the functions of the system. For this reason overwork has been credited with the power of producing anemia as well as many other serious systemic disturbances. However, it is rare to find an authentic case of anemia which can be traced directly to overwork without the mental influence of worry and the coincident nervous strain from this latter cause.¹

(4) Intestinal Parasites.

The anemia caused by intestinal parasites may be of such a severe grade as to resemble very closely the type of pernicious anemia. In many cases it is an impossibility to make a differential diagnosis between these types without the finding of an intestinal parasite which will clear up the diagnosis. In these cases the blood picture returns more or less quickly to normal after removal of the parasite in question, while in the pernicious anemia of unknown origin the progress of the disease is always toward a fatal termination. It is probable that the cause of the severe secondary anemia due to the presence of the intestinal parasite is a result of the toxic condition set up by the absorption of the hemolytic toxins elaborated by the parasite.² A very severe anemia of the secondary type is frequently seen as a result of decomposition of the intestinal contents and in cases of chronic constipation in which the direct toxic agent is at present unknown. The most common intestinal parasites causing the severe types of secondary anemia are (1) *uncinaria duodenale*, (2) *strongyloides intestinalis*, and (3) *bothriocephalus latus*. The first of these causes an anemia which is very closely related to that shown by miners and tunnel diggers, and seems to be much more prevalent in the southern part of the United States, although it occurs in many different countries. The blood count may fall below one million red cells and the hemoglobin may be as low as 15 per cent., while all varieties of degenerative changes may be seen in the erythrocytes. In the anemia following infection with the *bothriocephalus* we find very marked similarity with the primary pernicious type. One-half to two-thirds of the nucleated reds in this variety may be of the megaloblastic type and yet may disappear within two to three weeks after the worm has been expelled. It is uncommon to find in secondary anemias caused by the parasites above mentioned any marked eosinophilia, which is so common in cases of infection with many of the other forms of intestinal parasites. The anemia of the severer type seems to prevent a chemotaxis toward eosinophile cells.

¹ See Ash, Arch. Int. Med., 1914, XIV, 8.

² See Beumer, Biochem. Ztschr., 1919, XCV, 239; Schwartz, Jour. Agric. Res., 1919, XVI, 253; Arch. Int. Med., 1920, XXVI, 431.

(5) **Fever.**

It is still very much of a question whether the blood in febrile cases shows the characteristics of a secondary anemia, as the result of the temperature increase. It has been shown that increased temperature, in itself, does not always produce anemia, although we do have marked destruction of the red cells and a coincident loss in the amount of hemoglobin of the remaining cells. So great is the influence of the toxin of the febrile condition that it is highly probable that the anemia so frequently observed in febrile cases is due to a combination of causes, rather than to a specific effect of the increased temperature. The changes in the white cells in febrile cases are not always of the same character, the variations being dependent upon the specific causative factor of the fever. As the blood changes in the acute infectious fevers are of more or less importance, they will be discussed later under separate headings. The anemias which are secondary to both the acute and chronic infections are probably directly due to the influences of the toxins upon the blood and blood-forming organs. The condition of general nutrition as well as the state of digestion, especially in the chronic states, such as tuberculosis, leprosy, and syphilis, must be regarded as important factors in the causation of these secondary types.

(6) **Blood Poisons.**

There are a very large number of compounds which produce, when taken in toxic doses, very marked changes in the qualitative and quantitative composition of the blood. As is well known, iron compounds in therapeutic doses increase the amount of hemoglobin in the red cells and also increase the number of red cells up to a certain point. Many of the effects which are attributed to iron compounds may be due to the improved hygienic and dietetic conditions which usually prevail during the administration of these substances. Yet the therapeutic results following the administration of iron are such as to make it certain that a specific influence of this drug is present in anemic conditions, especially of the chlorotic type.

Many compounds produce a very marked secondary anemia, the most important of these being alcohol, opium, lead compounds, cocain, and acetanilid. While others, such as arsenic, nitrobenzol, nitroglycerin, phenacetin, and poisonous mushrooms, cause dissolution of the red cells with marked hemoglobinemia. The anemia following the use of lead, either in toxic doses or after its slow absorption from constant contact with it in the arts, is of great practical importance. The causes of this lead anemia are rather complex. The lead compounds have a direct action on the red cells and on the blood-forming organs as well as upon the gastrointestinal tract and the eliminative organs. While the anemia shows no especial characteristics as regards the number of red cells and the amount of hemoglobin, yet the peculiar granular degeneration and the polychromatophilia are sufficient to differentiate this type from most of the other secondary anemias. The basophilic degenerations of the reds is more marked in lead anemia than in almost any other condition and usually runs parallel to the severity of the clinical symptoms of

the case. In arsenical poisoning also we occasionally find a slight amount of granular degeneration of the red cells, but the hemoglobinemia in this latter condition will differentiate it from lead anemia.

(B) Leukemia.

Although acute forms occur, leukemia may be regarded as an essentially chronic condition which is characterized on the one hand by definite changes in the lymphatic and myeloid tissues of the body, and, on the other hand, by certain peculiar changes in the number and relations of the various cellular constituents of the blood. These latter conditions must be regarded as purely symptomatic of the preceding states and not as the direct pathological condition in themselves.¹ Leukemia has been classed as a primary anemia, although the changes in the blood are here more directly related to variations in the white cells than to characteristic changes in the red corpuscles; yet we do find a diminished red count as well as a diminution in the amount of hemoglobin in this condition. It is a disease marked by the constant presence in the blood of granular mononuclear or polynuclear cells, or an increase of the nongranular cells with round nuclei. While the leucocyte count is almost invariably increased to a marked extent, we find cases in which the number of cells is normal, but we find great deviations from the normal relations of these white cells.

While the tendency is becoming more and more general to regard this condition as a single entity, manifested by various blood pictures, yet we find the cells grouping themselves together in such definite ways that we are justified in dividing leukemia into three general types, with transitions from one to the other form.² These types are (1) splenomyelogenous leukemia or "myeloma," (2) lymphatic leukemia or "lymphemia," (3) mixed leukemia. Each of these types shows a distinct blood picture which permits of the classification of the condition studied.

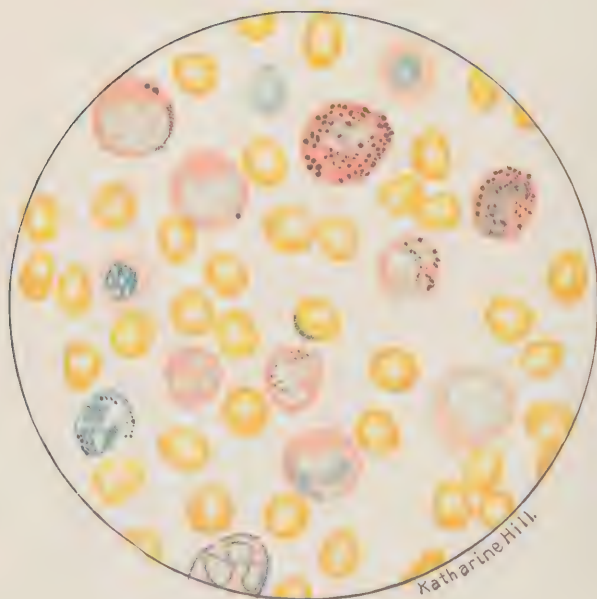
(1) Splenomyelogenous Leukemia.

This condition was formerly subdivided into two distinct types—the true splenic and the myeloid leukemia. However, practically none of the cases reported could be definitely classed under either one of these headings, as the blood picture was always referable to disturbance in both the spleen and marrow. This type of leukemia is characterized by a marked increase in all of the granular cells, especially of the neutrophile, eosinophile, and basophile types, while the nongranular cells are not so characteristically increased.

¹ It is probable that leukemia, both of the chronic and acute types, is of infectious origin. Streptococci and the bacillus hodgei have both been isolated from these cases. See Steele, Boston Med. and Surg. Jour., 1914, CLXX, 123; Simon and Judd, Jour. Am. Med. Assn., 1915, LXIV, 1630; Wilbur, Ibid., 1915, LXV, 1255; Dias, Brazil-Med., 1915, XXIX, 305, and 329; Ward, Brit. Jour. Child. Dis., 1917, XIV, 10.

² It is probably true that a classification of these types on purely morphological bases is futile. See von Bombard, Ztschr. f. klin. Med., 1914, LXXX, 506; Emden and Rothschild, Deutsch. Arch. f. klin. Med., 1914, CXV, 304; Pantou, Tidy and Pearson, Quart. Jour. Med., 1914, VII, 304; Rummo, Rif. Med., 1914, XXX, 807 and 926; Castellino, Ferrata and Rummo, Gazz. d. osp., 1914, XXXV, 1813; Rotky, Zentralbl. f. inn. Med., 1914, XXXV, 953; Martelli, Rif. Med., 1914, XXX, 1233, 1271, 1294 and 1324.

PLATE XXVI.



BLOOD IN SPLENO-MYELOGENOUS LEUKEMIA. (TRI-ACID STAIN.)

Gross Appearance.

The gross appearance of the blood is normal even though the leucocytes are increased to an enormous extent. In extreme cases it may appear pale and opaque and does not flow from a puncture as readily as normal blood. In making smears of such blood, the preparations appear granular and are not readily spread so that the future examination is rendered somewhat difficult.

Red Cells.

As a rule, these cells are diminished in number, but the oligocythemia is of a mild degree, the average count being about 3,000,000 although it may run as low as 1,500,000. As a general rule, the red cells diminish in proportion to the increase in the number of white cells. Occasionally we find cases in which an oligocythemia persists with a normal or slightly increased leucocyte count. Such a condition might lead to the diagnosis of pernicious anemia, unless the differential count was carefully studied. The red cells are usually pale and of the chlorotic variety. Very little degeneration of the red cells is observed, microcytes and macrocytes are rare, but a few poikilocytes are seen in practically all cases. Polychromatophilia is more or less common and cells showing basophilic granulations appear with more or less frequency. Normoblasts are very common in this condition, yet their absence does not rule out the diagnosis of leukemia. Megaloblasts and giantoblasts are frequently seen and are sometimes many in number, although they rarely if ever exceed the normoblasts in number.

Hemoglobin.

The hemoglobin is reduced to a somewhat greater extent than is the number of red cells, the color index being about 0.6 and the average percentage of hemoglobin about 40.

Leucocytes.

In this condition we find the leucocytes increased, as a rule, to a very marked degree, counts running as high as 750,000 having been seen by the writer. Osler gives as his average for the white count 298,700, while the average may vary to a slight extent at different periods of the day. Some of the cases show a uniformly high count, others a moderate count and a few others a low count of about 100,000 cells. It is this increase in the number of white cells which gives the blood its peculiar opacity in this condition and may make a diagnosis possible by mere inspection. In some cases of leukemia we find a normal count of white cells while in others the count may be similar to that of a simple leucocytosis. It is the differential count in combination with the large increase in cells which should be considered characteristic, rather than a simple increase in itself.

Differential Count.

Neutrophile Myelocytes.

These cells are large mononuclear cells with neutrophile granules. They are present in large numbers, averaging about 35 per cent. of all the leucocytes present. A diagnosis of leukemia is almost always possible when we have

such an extreme neutrophile myelocytosis along with an extreme leucocytosis. These myelocytes appear in two forms: (1) the large myelocytes of Cornil, which may be as large as 30 microns in diameter and have a large, pale, eccentric nucleus which is poor in chromatin. These cells are seen only in splenomyelogenous leukemia and in some of the secondary leukemias of children and must be regarded as practically pathognomonic of this condition.¹ (2) Small myelocytes about the size of the normal polynuclear leucocytes with a centric, round nucleus staining deeply with the various aniline dyes. We find all gradations between these large and small myelocytes, sometimes observing a few which are about the size of the red cell. The granulations of these cells are sometimes numerous, but may be entirely lacking so that they may be indistinguishable from the large lymphocytes unless the pale quality of their nuclei is remembered. The degenerative changes in these myelocytes are few in number and are limited, as a rule, to the hydropic form usually seen in chlorosis.

Polynuclear Neutrophile Leucocytes.

These cells are relatively diminished, their average, according to Cabot, being about 46 per cent., although an absolute increase is present, amounting to as much as 60,000 to 75,000 cells. Marked variations in the size of these cells are common, some of them being very large, some very small and no definite relation existing between the numbers of the large and small cells. These variations in size of the polymorphonuclear cells are rarely if ever seen in ordinary leucocytosis. It is very common to find cells with irregularly shaped nuclei and with more than one form of granule, which may vary in tint depending on the method of fixation. Marked degenerative changes in these cells are very common. Thus we find their stickiness is increased, their nuclei usually pale and frequently showing karyokinetic figures. All grades of variations in the granulations may be observed, the granular cytoplasm being occasionally replaced by a homogeneous highly refractive material.

Eosinophiles.

The eosinophile cells are usually much increased in the splenomyelogenous form of leukemia, but their percentage relations to the other leucocytic forms are practically normal. Their number may run from 3,000 to 100,000 the average absolute number being about 12,000, while their percentage is about five. The total number of these cells per cmm. greatly exceeds that found in any other condition so that we accept, with Ehrlich, such an increase as pathognomonic of splenomyelogenous leukemia. These cells appear in all modifications, some of them being very small, while some of them are very large. The eosinophile form of myelocyte occurs in large numbers, but never is as numerous as is the neutrophile myelocyte. We occasionally observe all forms of transition between the myelocyte and the eosinophile leucocyte, the eosinophile myelocytes occasionally forming the majority of the eosinophile cells. The granulations of these cells may be of uniform size and staining quality or there may be some basophile granulations among the eosinophiles

¹ See Klein, *Deutsch. med. Wchnschr.*, 1913, XXXIX, 2513.

while the granulations themselves may vary greatly in size. Ewing considers eosinophile myelocytes with granules of unequal size and density of stain as pathognomonic of myelocythemia.

Basophiles.

According to Ehrlich, we always find an increase in the number of most cells in leukemia, the absolute increase being in some cases greater than that of the eosinophiles and is always proportionately higher. This increase is so marked as to constitute a very reliable diagnostic feature of the blood. The number of basophiles may run as high as 140,000 (Taylor), while the percentage may vary from 5 to 47.

Lymphocytes.

The number and proportions of lymphocytes in the splenomyelogenous leukemia vary in different cases and at different times in the same case. As a rule, their percentage is reduced averaging about 10, while an absolute increase is usually present, this increase having no uniform relationship to the stage or character of the disease. These cells vary much in size, the large cells usually outnumbering the small ones. Large mononuclear cells with very faint cytotreticulum and vesicular nucleus occur in large numbers in this form of leukemia and seem to have no special significance, although they may be mistaken for the large lymphocyte or for the myelocyte. Degenerative changes are observed in both the small and large lymphocytes in leukemia; thus the nuclei of the small cells may become incurved and bilobed or even trilobed, while the cell body remains basophilic (Rieder).

Points in Diagnosis.

An excessive leucocytosis, with a large proportion of neutrophile myelocytes, the presence of a large number of eosinophile myelocytes and of basophile cells, the presence of atypical cells, both of the mononuclear and polynuclear variety, and large numbers of nucleated red cells are the chief characteristics. Any one of these points may fail for a time, but will usually be evident at some stage of the disease. The large size of the myelocyte is much more characteristic than the mere presence of these cells, so that we should confine our diagnosis to cases which show irregularity in size, staining qualities, and degenerative reactions of these cells rather than to those showing merely an increase. The presence of the large number of eosinophiles, especially those showing granules of irregular size and staining qualities, is a very important point to be remembered in the diagnosis of this condition.¹

(2) Lymphatic Leukemia (Lymphemia).

In this form of leukemia we observe a marked increase in the number of mononuclear nongranular cells in distinction from the previous form of leukemia in which the increase is rather in the number of the granular types. While a variety of the mononuclear nongranular cells are present, there is usually observed a predominance of one particular form and size, in some

¹ See Diebald and Entz., *Folia Hæmat.*, 1913, XV, 59; also, Ghon and Roman, *Ibid.*, 72, and Milne, *Jour. Am. Med. Assn.*, 1913, LX, 822. Goodall (*Boston Med. and Surg. Jour.*; 1914, CLXX, 789) discusses the nitrogenous metabolism in this condition; Tiberti, *Sperimentale*, 1918, LXXII, 482; Sweet, *Southwest. Med.*, 1919, III, 19.

cases the small mononuclear cell with a narrow ragged rim of protoplasm, in others the cells of the large lymphocyte type, and in others large cells whose protoplasm is basophilic or in some cases distinctly acidophilic.¹

Red Blood-cells.

In this form of leukemia we find a much greater anemia than in the splenomyelogenous form, although we may observe a normal red count for some time. The number of cells varies between 1,500,000 and 4,000,000, while the average percentage of hemoglobin is about 37 per cent. Nucleated red cells are rare in this condition, yet in the severer cases we may find them as numerous as in the splenomyelogenous type. All forms of degeneration noted under the previous type of leukemia are occasionally seen in this latter form.

Leucocytes.

The leucocytes are, as a rule, increased, the average being about 145,000, according to Osler. In this form we may find aleukemic periods which may last for a considerable period of time, the count usually rising just before death.

Differential Count.

According to Grawitz the cases of lymphatic leukemia may be divided into (1) those in which the increase of leucocytes is especially in the small mononuclear variety, (2) those showing an increase in the medium-sized cells with basophilic homogeneous protoplasm, and (3) those in which the cells which predominate are very large and usually degenerated. All these forms may occur together and may vary in the same case at different times. These mononuclear cells may constitute as high as 99 per cent. (Osler) of the total number of leucocytes. These leucocytes show in a very large number of cases much degeneration either of the protoplasm or of the nucleus, very few of the cells showing mitosis which is so common in the splenomyelogenous form. In this type of leukemia polymorphonuclear cells are rare, eosinophiles usually absent, and myelocytes and basophiles rarely if ever present. This type of leukemia is not easily amenable to diagnosis, especially in differentiating it from some cases of sarcoma in which the blood may show a similar picture.

In some cases we find a lymphatic leukemia with a considerable number of myelocytes both of the eosinophile and neutrophile type. This has led to the differentiation of a "mixed leukemia," which does not seem to be advisable as we may find myelocytes in the pure lymphatic type of this disease.

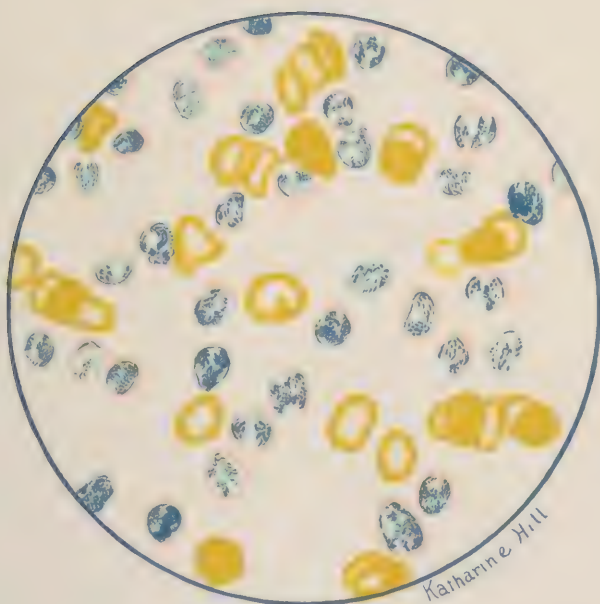
(3) Acute Leukemia.

This form of leukemia is characterized by its brief course (from six to eight weeks), by the severity of its symptoms, the frequency of the hemorrhagic diathesis, rapidly developing cachexia, and death. This condition occurs chiefly in young people and is usually of the lymphatic type,² although

¹ See Nakamura, *Deutsch. Ztschr. f. Chir.*, 1914, CXXXII, 275; Jackson and Smith, *Boston Med. and Surg. Jour.*, 1915, CLXXII, 130; Fleischmann, *Folia Hæm.*, 1915 XX, 17; Suter, *Cor. Bl. f. Schweiz. Aerzte*, 1915, XLV, 1281; King, *Bull. Johns. Hopk. Hosp.*, 1917, XXVIII, 114; Packard and Ottenberg, *Jour. A. M. A.*, 1917, LXVIII, 954; Du Bray, *Am. Jour. Med. Sc.*, 1922, CLXIII, 430; Fineman, *Arch. Int. Med.*, 1922, XXIX, 168.

² See Strauch (*Am. Jour. Dis. Child.*, 1913, V, 43) for a discussion of this condition in children. Also, Beltz, *Deutsch. Arch. f. klin. Med.*, 1913, CXIII, 116; Ballagi, *New York Med. Jour.*, 1914, XCIX, 68; Yamakawa, *Mitt. a. d. Med. Fak. Tokyo*, 1914, XI, 115;

PLATE XXVII.



LYMPHATIC LEUKEMIA. (TRI-ACID STAIN.)

a few cases of the myelogenous variety have been reported (Billings and Capps).¹ In all cases the anemia is extreme, the red cells usually running below 1,000,000 and the hemoglobin as low as 10 per cent. There is no type of cell which is characteristic of this form, although the cells are much more uniform in size than in the chronic states of this disease. In some cases nearly all of the cells have a basophile protoplasm, while in others they show acidophilic properties. Nucleated reds are usually rare, although they may be present in fairly large numbers. The drop in the count of red cells is usually sudden and denotes rapid blood destruction. The leucocyte picture resembles closely that of acute infections.

(C) Pseudoleukemia.

Under the heading Pseudoleukemia have been grouped a great variety of diseases, which have in some cases the external appearances of the disease, such as the glandular swelling, splenic tumor and progressive cachexia without, however, showing the blood picture which is so characteristic of leukemia. On the other hand, we find conditions which have little in common with the clinical findings of leukemia and yet show a blood picture similar in some respects to that of leukemia. It is difficult to group all of these cases under one heading, as the blood picture is not characteristic for any one of these conditions.

(1) Hodgkin's Disease.

This condition, first described by Hodgkin in 1823, is characterized by chronically progressing cachexia with enlargement of the lymph-glands and spleen. It has been called, synonymously, lymphatic pseudoleukemia, lymphosarcoma, malignant lymphoma, lymphatic anemia and aleukemic myelosis.² It is probably infectious in nature, the etiologic factor being probably the *Bacillus hodgkini* (see Chap. XI). The blood characteristics in this disease are more particularly those of a true cachexia, the red cells showing a diminution in the number which may be as low as 2,200,000, but which is usually between 3,000,000 and 4,000,000. The more severe and pronounced the signs of anemia and cachexia the lower the number of cells, the number in these cases running as low as 1,500,000. Morphologically, the red cells show much less deviation from the normal than in other severe anemias, the average size of the cell being usually normal, degenerations of the red cells appearing only in the very severe conditions, microcytes and

Citron, Berl. klin. Wchnschr., 1914, LI, 332; Deutsch. med. Wchnschr., 1914, XL, 629; Brinckmann, Norsk Mag. f. Laegevid., 1915, LXXVI, 1473; Stein, Med. Record, 1916, XC, 147; Gasbarrini, Policlinico, 1919, XXVI, 906; Sternberg, Wien. klin. Wchnschr., 1920, XXXIII, 553; Smith, Am. Jour. Dis. Child., 1921, XXI, 162; Ceyon and Lavedan, Bull. de la Soc. Méd. des Hôp., 1921, XLV, 106; Brousselle, Ibid., 205; Brousselle and Fiessinger, Ibid., 211; Kaltenbach, Arch. des Mal. du Cœur, 1921, XIV, 156; Toledo y Valere, Siglo Med. 1921, LXVIII, 420; Paiseau and Alcheck, Bull. de la Soc. Méd. des Hôp., 1921, XLV, 1424; Clerc, Ibid., 1542; Bloedorn and Houghton, Arch. Int. Méd., 1921, XXVII, 315; Ewald, Frehe and Hennig, Deutsch. Arch. f. klin. Med., 1922, CXXXVIII, 353.

¹ See Beifeld, Arch. Diag., 1915, VIII, 244; Marshall, Arch. Int. Med., 1915, XVI, 1045; Sappington, Am. Jour. Med. Sc., 1916, CLII, 238; Knox, Am. Jour. Dis. Child., 1916, XI, 462; Ross, Lancet, 1916, II, 940; Simon and Rosenthal, Jour. A. M. A., 1917, LXIX, 2168; Theo., Jour. Med. Res., 1918, XXXVIII, 385. Aubertin and Giroux, Presse Med., 1921, XXIX, 314.

² See Hirschfeld, Ztschr. f. klin. Med., 1914, LXXX, 126; Ghedini, Gazz. d. osp., 1914, XXXV, 2037; Ibid., 1915, XXXVI, 1, 33, and 503; Ragusin, Sem. Med., 1915, XXI, 57; Chosrojeff, Folio Hæm., 1915, XX, 33; Block, Ugesk. f. Laeger, 1916, LXXVIII, 831.

macrocytes, as well as nucleated erythrocytes, being very unusual except in the late stages. The hemoglobin content runs parallel to the number of red cells, being the lowest in those cases showing very low counts. The leucocytes are slightly increased, averaging about 12,000. This failure of a leucocythemia enables us to differentiate Hodgkin's disease from a true lymphemia. The differential count of the leucocytes may show a relative lymphocytosis, the relation of the lymphocytes to the polynuclear cells being as three to one instead of the normal one to three.¹ Ehrlich and Pinkus consider this relative increase of lymphocytes characteristic of true pseudoleukemia in contradistinction to sarcomatous and other lymphomatous conditions. According to Grawitz, an increase in the leucocytes is associated with an unsatisfactory course of the disease, while a diminution in the number is observed as the disease progresses toward convalescence.

Cases have been reported which would seem to point to the transition of Hodgkin's disease into a true leukemia, so that we may find irregular progress of a pseudoleukemia as an evidence of a transitional stage.

(2) Tuberculosis of the Lymph-glands.

Why this condition has been classed as a pseudoleukemia is questionable, as the blood picture shows nothing beyond a secondary anemia with cachexia or may show even a normal red and white count. A large increase in the number of leucocytes, which is so characteristic of leukemia, is rarely seen, although a true leukemia may arise in the course of a glandular tuberculosis. The differentiation of this condition should be based upon examination of an excised gland, which will show distinct tuberculous lesions and usually will contain the tubercle bacilli in demonstrable numbers.² In other cases we may find a simple lymphoid hyperplasia without any distinct inflammatory changes and without demonstrable bacilli. Such nodes should be tested by inoculation experiments as advised by Ewing. Heredity plays a great rôle in the diagnosis of these tubercular conditions, while a scrofulous child should always be looked upon with suspicion. A splenic tumor appearing coincidentally with the glandular swelling would speak rather against tuberculosis and in favor of a lymphatic pseudoleukemia. The diagnosis of this condition by examination of the blood alone is a practical impossibility. It is this type of case that is especially amenable to diagnosis by the use of the various tuberculin tests.

(3) Lymphosarcoma.

The lymphosarcomata usually run their course either as primary benign lymphomata or as the malignant sarcomata. The blood findings in these conditions show nothing beyond a slight anemia with nothing characteristic

¹ Bunting (Bull. Johns Hopkins Hosp., 1911, XXII, 114 and 369) considers an increase in the number of transitional cells together with increase of blood platelets as quite characteristic of true Hodgkin's disease. See, also, Bunting, Bull. Johns Hopkins Hosp., 1914, 173 and 177, Yates, *Ibid.*, 180; Cunningham, *Am. Jour. Med. Sc.*, 1915, CL, 868; Mellon, *Ibid.*, 1916, CL, 704; Moore, *Jour. Infect. Dis.*, 1916, XVIII, 569; Woolley, *Jour. Lab. and Clin. Med.*, 1917, II, 523; Keuper, *Deutsch. Arch. f. klin. Med.*, 1919, CXXX, 118; Blankenhorn and Goldblatt, *Jour. Am. Med. Assoc.*, 1921, LXXVI, 583; Fox and Farley, *Am. Jour. Med. Sc.*, 1922, CLXIII, 313.

² Resort should be made to the antiformin method, if ordinary staining processes are not conclusive.

in the appearance of the white cells. The diagnosis must be based entirely on the examination of the excised gland or tumor.¹

(4) **Gummatous Lymphoma.**

An exact diagnosis of this syphilitic swelling of the lymphatic glands is at the present time a matter of more or less difficulty. The previous history, as well as other manifestations of syphilis, must be studied and a careful search made for the presence of the spirochæta pallida. It is to be said that these organisms have been frequently reported in lymphatic enlargements which seem to have no direct relationship to purely syphilitic conditions. The interpretation of one's findings is of the utmost import as artefacts, which commonly appear in preparations of broken-down glandular tissue, resemble very closely the spirochæte. The blood condition shows nothing characteristic and can have only incidental diagnostic importance. Application of the Wassermann serum test might throw much light on the diagnosis.

(II) **General Pathology.**

(a) **Blood Changes Following Surgical Intervention.**

Under this heading the writer will not attempt to take up all of the surgical conditions, as the vast majority are not associated with any direct hematological characteristics.

As a rule, it may be said that pus formation anywhere in the system will cause a leucocytosis. The degree of this leucocytosis averages about twice the normal standard, but may greatly exceed this figure in individual cases. It must be said, however, that trivial as well as extensive pus formations may be accompanied by normal or even subnormal values for the leucocytes. This is due to the facts that small pus foci do not cause any systemic reaction and extensive pus formation may overcome the power of the system to react against the infection. If the pus cavity is well encapsulated, the absorption of the toxin from this focus is necessarily limited so that we may find no leucocytosis, even though a very large pus cavity is present. Thus we find in localized peritonitis following appendicitis that a leucocyte count may be normal, but may suddenly increase to a marked extent as an indication of the rupture of the cavity and an extension of the process.² A general rule is that a distinct increase in the number of cells in excess of the figure originally obtained is indicative of the extension of pus formation and should put the surgeon on his guard as to operative interference.

If the absorption of toxic material from a focus of pus formation is great enough to produce a systemic effect upon a patient as manifested by a high leucocyte count, we find an anemia characterized by a marked diminution in the hemoglobin and the number of red cells, which is parallel in intensity to the severity of the poisoning.

Cases are frequently found in which a low leucocyte count prevails, although clinical evidence of severe sepsis is at hand. In these cases a differ-

¹ See Oliver, Jour. Med. Research, 1913, XXIX, 191; Steiger, Berl. klin. Wchnschr., 1913, L, 2129; Glanzmann, Deutsch. Arch. f. klin. Med., 1915, CXVIII, 52; Warfield and Kristjanson, Am. Jour. Med. Sc., 1916, CLII, 222. Hirsch (Trans. Chic. Path. Soc., 1920, XI, 76) reports a case of lymphosarcoma in which the blood changes might be confused with those of a leukemia or a severe primary anemia.

² See Friedman, Am. Jour. Med. Sc., 1914, CXLVIII, 540.

ential count of the leucocytes should be made in all cases, as the low leucocyte count may throw one off his guard unless this precaution be taken. Should the polynuclear neutrophiles form 80 to 95 per cent. of the total leucocytes, a severe infection is indicated, even though the leucocyte count may be subnormal.

If a leucocyte count does not diminish or even return to normal within one to two days after operation, this should be taken as evidence that a reinfection has occurred or that the pus cavity has not been properly drained. Recourse must, therefore, be had to measures to overcome the secondary infection.

It has been found that administration of ether and chloroform causes a leucocytosis which usually lasts from 24 hours to 48 hours and which may interfere with the interpretation of a blood examination. This point must be borne in mind in the examination of blood of cases which have shown high leucocyte values prior to operative procedure. The differential count in such cases, however, will show only slight variations in the proportions of the different types of cells so that one may judge as to the cause of the secondary leucocytosis by determination of the polynuclear cells. The number of red cells as also the amount of hemoglobin are very markedly reduced in some cases following the administration of an anesthetic, so that a direct secondary anemia may be the result. This fact has led to the refusal by many surgeons to resort to operative procedure in cases which show as low as 30 per cent. hemoglobin prior to operation.¹

As the blood changes in surgical conditions, which are not accompanied by pus formation, are not especially characteristic and are more especially associated with the diseases of the special organs, the writer will refer such discussions to other headings.

(b) Blood in Constitutional Diseases.

(1) Diabetes Mellitus.

In diabetes mellitus the changes in the cellular content of the blood are not very marked. The leucocytes may be subnormal, normal, or slightly increased, usually a very slight leucocytosis being observed. The amount of hemoglobin is usually reduced to a very slight extent, while the number of red cells may be slightly increased.

One of the most striking peculiarities of the blood in diabetes is the presence of an excess of fat (lipemia). Microscopic examination usually reveals the presence of the extracellular globules, but in some cases fat is present in sufficient amount to permit of demonstration by macroscopic methods. Glycogen has been found both in the plasma and in the leucocytes of diabetic blood and shows the peculiar characteristics discussed in the section on Iodophilia (p. 605). Certain peculiarities of the blood in diabetes have led to the introduction of tests supposed to be characteristic for such blood. These tests are occasionally of diagnostic value, although a diagnosis may, as a rule, be made even when these tests do or do not obtain.

Bremer's Test.—This test is based upon the fact that diabetic blood does

¹ See Cullen, Surg., Gynec. and Obst., 1913, XVII, 276.

not stain to any appreciable extent when treated with certain anilin dyes. Thick smears of the blood are made upon slides and are fixed by dry heat. These smears are then covered with a 1 per cent. aqueous solution of Congo red and allowed to stain for a few minutes, after which they are rinsed in water and dried. Diabetic blood will be stained either a faint yellow or not at all, while normal blood will be colored a bright red. A 1 per cent. solution of Biebrich scarlet will stain the diabetic blood intensely while the normal blood is unstained. Bremer's original staining solution was made up as follows: Saturated watery solution of eosin and of methylene blue are mixed in equal proportion when a precipitate forms which is filtered, washed, dried and powdered. To 24 parts of this powder are added six of powdered methylene blue and one of eosin. One-twentieth of a gm. of this mixed powder is dissolved in 10 c.c. of 33 per cent. alcohol and forms the staining solution in which the specimens are stained for four minutes. The diabetic blood stained by this solution has a greenish tint, while normal blood is reddish violet.

Similar reactions have been found in normal blood, in leukemia, in exophthalmic goiter, in Hodgkin's disease, in multiple neuritis, and in some cachectic conditions, but the reaction in all these cases is very inconstant. According to Bremer, cases of renal diabetes do not give this reaction, which is more characteristic of the pancreatic type of the disease.

Williamson's Test.—This test is performed as follows: Two drops of blood (20 cmm.) are dissolved in four drops (40 cmm.) of water and to the solution is added 1 c.c. of a 1 to 6,000 aqueous methylene blue solution. To this is added four drops (40 cmm.) of 6 per cent. solution of liquor potassæ and the test-tube placed in boiling water for four minutes. Diabetic blood will decolorize the solution, while normal blood leaves it a deep blue. The same effect is observed by using diabetic urine instead of the blood.

(2) Gout.

Little information is available as to the variations in the number of the red and white cells in the gouty condition. The recorded observations show that acute gout has little effect upon the number of red cells and upon the amount of hemoglobin, while chronic gout may be accompanied by an anemia which is more directly referable to causes other than the gouty condition itself. The leucocytes are usually increased in the acute attack, while in the chronic form the leucocytosis is of a more moderate grade. Neusser in working upon the blood of gouty patients found many polymorphonuclear leucocytes whose nuclei were surrounded by basophile granules—the so-called perinuclear basophile granules. These he considered diagnostic of the uric acid diathesis, but Fitcher and Simon have found them in many other conditions, while Ehrlich regards them as artefacts.

While the chemistry of the blood in gout has been the subject of much investigation for a long period, nothing of diagnostic importance has been found in the chemical properties of the blood. The excess of uric acid has been shown not to be pathognomonic of gout, as it is present in many other conditions which are clinically far removed from the gouty state.

(3) Addison's Disease.

This disease is usually associated with a severe grade of anemia, the number of red cells being reported as low as 1,120,000 while the percentage of hemoglobin is coincidentally reduced. The leucocytes are usually diminished, but may be slightly increased, while the relative proportions of the different cells are not markedly changed. As the disease progresses unfavorably, a relative lymphocytosis may be observed, but this is not always the case.¹

(4) Rickets.

The state of the blood in rickets varies with the extent and severity of the primary disease and is markedly affected by complications. Cases are reported in which the red cells are practically normal and in which the hemoglobin was only very slightly reduced. This disease is not associated with any special type of anemia, although the hyperemia of the bone-marrow might be expected to yield a large number of nucleated red cells. The usual condition of the blood in rachitic children is of the type of simple chlorotic anemia. A grave secondary anemia is seen in many cases in which there are serious complications. The leucocytes in practically all cases of rickets are increased, but may not exceed the normal limits for the child. As is usual in the blood of a child, the lymphocytes are increased while the eosinophile cells are often relatively numerous.² Just exactly what the cause of the leucocytosis in rickets is must be left to the realm of hypothesis, as neither the gastroenteritis nor the hyperplastic splenitis are sufficient to explain all cases.

(5) Myxedema.

In this disease we usually find an anemia of the secondary chlorotic type along with a moderate leucocytosis. The number of cells is usually somewhat diminished, although their size is usually increased. The proportion of the different leucocytes does not vary, although a few myelocytes are sometimes seen in the blood, which is numerically normal in other respects.

Although many studies of the chemistry of the blood in myxedema have been made, little knowledge has been forthcoming as to the exact cause of this toxemia. It is highly probable that the changed activity of the thyroid gland in this disease influences other organs to such an extent that slight anemia is the result and we should, therefore, assume that this anemia is more a secondary one than a primary result of thyroid insufficiency.³

(c) Blood in Acute Infectious Diseases.

In a study of the blood in acute infectious diseases, we must remember that there are certain general rules which apply to all of such diseases with a very few exceptions. The interrelation of the fever with the resistance of the system in general is so close that it is hard to say in any given case whether certain changes in the blood are or are not due to the increased temperature in itself. There can be little doubt that a high temperature working over a

¹ See Buettnermüller, *Med. Klin.*, 1914, X, 1354; Engelsmann, *Folia Hæmat.*, 1915, XIX, 333; Scheltema, *Nederl. Tijdschr. v. Geneesk.*, 1915, I, 1767.

² See Ostrowski, *Folia Hæmat.*, 1912, XIII, 305.

³ See Salvatore (*Riforma Med.*, 1913, XXIX, 1373) for a discussion of the blood changes in exophthalmic goiter.

considerable period of time will destroy large numbers of red cells and will bring on various changes in the blood which might be misinterpreted. A rather extensive concentration of the blood along with a progressive loss of albumin is observed in practically all conditions associated with fever. We should, therefore, expect to find the number of red cells increased at the outset of such condition, owing to the concentration of the fluid portion, while a distinct anemic condition may become evident only after the lapse of some time.

Fever in itself does not have a large influence upon the number of leucocytes, but it may be stated as a general rule that most infectious diseases (the exceptions being malaria, typhoid, tuberculosis, influenza, and measles) are associated with an increase in the number of white cells. This is not an invariable rule, as will be seen under the discussion of the various infectious types. The leucocytosis so commonly seen associated with infection is no doubt due to the action of the bacteria themselves and of their products upon the leucocytes. The positive chemotaxis which bacteria and their toxins exert upon the leucocytes is very marked. Moreover, as the blood becomes laden with these abnormal products, new leucocytes are thrown into the circulation to aid the old ones in their phagocytic action. Just what substances are accountable for the increased opsonic power of the serum in any specific infection must be left undecided for the present.

In the following discussion of the various infectious diseases, the writer will not attempt to give more than a brief discussion of the blood changes in these separate conditions, leaving associated questions to other writers.

(1) **Pneumonia.**

This disease is, hematologically, one of the most definitely characterized of all the infectious diseases. While the physical findings of this condition are largely local, the systemic effects are so marked that definite changes are seen in the blood both in the early and in the later stages of the infection. While showing so many characteristic findings in the blood, it is, at the same time, one of the most obscure in its relations to opsonins and to phagocytosis. Just why the virulent pneumococci should be so little capable of phagocytosis and why the addition of attenuated cultures of pneumococci or of extracts of virulent organisms should increase this phagocytic power of the leucocytes is at present very uncertain. We must, therefore, leave the discussion of this phase of pneumonia as well as of other infectious diseases to the section on Bacteriology of the Blood.

In pneumonia we find in the early stages that the blood is somewhat concentrated owing to the action of the increased temperature, while this concentration gradually increases as the exudate forms. Such a condition can lead only to an increased count of both the red and the white cells. As the disease progresses, the number of red cells shows a slight but a steady decline, which points not only to a destruction of the red cells, but to a diminished formation. This decrease in the number of red cells is occasionally seen only at the time of crisis, while in the cases in which the diminution is gradual the

period of diminution does not usually exceed ten days. It will be seen, therefore, that the red cells in pneumonia may be about normal in number and at the same time an anemia may be present which becomes evident only after the disease has progressed for some time. The red cells are, as a rule, normal in appearance, but an occasional polychromatophilic cell may be seen, especially in the severe cases. Rarely normoblasts may be observed and very rarely megaloblasts.

The hemoglobin usually shows a greater reduction than does the number of red cells, which decrease may become evident only after the fever has subsided. A reduction in the hemoglobin below 60 per cent. is very unusual in pneumonia of the pure type.

Pneumonia is one condition in which the leucocyte count may prove of great value. A leucocytosis appears in most of the cases, being absent in very mild cases as well as in those very severe ones which show very feeble resistance of the organism toward invasion by the pneumococci.¹ Rieder's observations are very interesting on this subject. He has found that the leucocytosis of pneumonia is more a function of the intensity of the infection and the degree of resistance toward this infection than it is of the fever or of the extent of the exudate.² A leucocytosis which may reach 12,000 to 20,000 appears very early in the course of this disease, and is usually evident at the time of the chill or immediately following. A steady increase is sometimes observed in the number of the white cells so that the maximum is usually reached just before the crisis. It is to be said that rapid extension of the disease as well as continuous high temperature may cause much irregularity in the count, cases being reported in which the leucocytes are high at first and steadily diminish as the patients grow worse. Others may show a sudden increase in the number of cells as the time of crisis is approached.³ According to Ewing, when the leucocytes increase slowly they usually diminish slowly and the disease defervesces by lysis. The degree of leucocytosis in pneumonia may reach any stage between the normal figure and that of 115,000 as reported by Laehr.

The increase in the number of white cells in pneumonia is largely referable to increase in the number of the polynuclear neutrophiles, these cells constituting as high as 97 per cent. of the total number of white cells. Associated with this polynuclear leucocytosis we have a marked diminution of lymphocytes, while the large mononuclear cells usually persist in considerable numbers. The eosinophile cells are always much reduced at the height of the leucocytosis, so that we may not be able to find a single one after very prolonged search. Cabot has reported a case in which the lymphocytes constituted 66 per cent. of a total of 94,600 white cells, but such a finding is not the usual one following infection with the pneumococcus. As defervescence goes on the polynuclear cells diminish very rapidly, while the lymphocytes increase and the large mononuclear leucocytes become very numerous, reaching as high as

¹ See Hess, *Am. Jour. Dis. Child.*, 1914, VII, 1.

² See Kline and Winternitz, *Jour. Exper. Med.*, 1913, XVIII, 50.

³ Dick (*Jour. Infect. Dis.*, 1912, X, 383) shows that proteolytic ferments develop in the blood about the time of crisis.

16 per cent. in a case reported by Türk. The eosinophile cells usually appear about the time of crisis, but occasionally their appearance is postcritical. The degenerative changes seen in the leucocytes in pneumonia are in no way different from those observed in other infectious diseases.

It will be noted, from the above remarks, that the blood changes in pneumonia are those of a mild anemia associated with a high-grade polynuclear leucocytosis and a distinct lymphopenia. Too much reliance must not be placed on the blood finding in a case of pneumonia, owing to the fact that many abnormal cases are present and show results different from the above which can be interpreted only by a complete study of the complications in any special case. As a general rule, it is to be said that an absence of leucocytosis is strong negative evidence against pneumonia, while leucocytosis may serve to differentiate this condition from typhoid fever and malaria with which it might be confounded, especially where the systemic and cerebral symptoms are more pronounced than are the local pulmonary changes.

(2) Typhoid Fever.

This condition, like the preceding, is very often associated with such marked systemic disturbance that the local intestinal manifestations are obscured and the diagnosis rendered somewhat difficult. While typhoid fever is a purely infectious condition and subject to the ordinary laws governing such cases, yet we find for some reason that the invasion of the blood by the specific organism is not associated with a leucocytosis, although the febrile rise may be very marked. The study of the characteristic serum reaction for typhoid fever as well as of the bacteriology of the blood must be left for a later section, the discussion here being limited to the changes in the microscopic appearances of the blood.

In this condition we find the total volume of blood very much diminished in the early stages, both as a result of the high temperature and the diarrhea and repeated hemorrhages which may occur at any stage of the disease. This concentration of the blood leads to an initial polycythemia which may last for two or even three weeks. However the characteristic change in the red cells is one of a slight and gradual decline, the number of these cells not usually falling below 4,000,000. One must be on his guard in an examination of the blood in any infectious disease lest he conclude from a slightly increased count that no anemia is present. It is a very safe precaution, although rarely followed, to determine the specific gravity of the blood so that one may compute the degree of concentration. In this way he may be able to show that the number of cells normally present in such a concentrated blood is much higher than in the case of suspected typhoid fever which he is examining. At any rate, it is wise to make frequent determinations of both the number of cells and of the hemoglobin, as a reduction in both of these elements takes place gradually as in the cases reported by Thayer and Da Costa.

The reduction in the amount of hemoglobin is in some cases very marked, being as low as 50 per cent. in one case observed by the writer. The repeated hemorrhages which so often occur in typhoid fever may cause marked varia-

tion in this value. The morphological changes which occur in the red cells are not very marked, as a rule, but may be very severe in case much blood is lost by frequent hemorrhages. Polychromatophilia is more or less frequent and irregularity in the size of the red cells is occasionally seen, while nucleation of these cells along with formation of a few megaloblasts may occur in severe hemorrhagic cases.

The leucocytes are usually normal in number in the early stages of uncomplicated cases, but any complication may cause a polynuclear leucocytosis which may be confusing to the worker. The behavior of the leucocytes is very variable in the early stages, so that one should never rest his diagnosis of typhoid fever upon a negative leucocytosis. As the disease progresses, the leucocytes show a gradual reduction, especially in the number of the polynuclear cells, which reduction continues until the disease has reached its highest point, after which they slowly increase.¹

The more severe the action of the typhoid toxin the lower is the leucocyte count, the reduction not usually going below 2,500 cells, the majority of cases showing a count between 4,000 and 6,000. It is not an uncommon thing to observe a leucocytosis during the later course of typhoid fever and it is not always easy to explain such a condition. Marked hemorrhage, cold baths, severe diarrhea, and usually perforation may account for the increase in the number of cells, but we do not always find a leucocytosis after such conditions.

In typhoid fever we find quite characteristic changes in the relations of the various types of leucocytes. During the first week the neutrophile cells do not, as a rule, increase, while the lymphocytes, especially of the medium-size variety, show a progressive rise. The lymphocytes are rarely below 25 per cent. of the total number of cells and may reach as high as 65 to 70 per cent. The eosinophile cells are usually low in number during the febrile period, but reappear about the time of defervescence.

It will thus be seen that the characteristic changes in the blood of typhoid fever are a slight anemia, together with a leucopenia and a relative, and in some cases absolute, lymphocytosis. Such characteristics are the usual ones of typhoid fever, but it is to be remembered that suppurative processes do not always produce a leucocytosis, nor is a leucopenia always present in typhoid fever.

(3) Scarlet Fever.²

In this condition we find the usual effects of fever manifested in a slight concentration of the blood, leading in the early stages to a polycythemia. The usual change, however, in the red cells is one of gradual reduction in numbers to as low as 3,000,000 cells and occasionally much lower. The hemoglobin also suffers quite a diminution, so that the anemia may reach quite a severe grade.

The leucocytes in scarlet fever usually increase in number one or two

¹ See Ravenna, *Rif. Med.*, 1917, XXXIII, 65 and 93.

² See Koessler (*Jour. Am. Med. Assn.*, 1912, LIX, 1528) for a discussion of the recent advances regarding scarlet fever. Mallory and Medlar, *Jour. Med. Res.*, 1916, XXXIV, 127.

days before the appearance of the rash and continue to increase until quite a marked leucocytosis, ranging from 10,000 to 50,000 cells, becomes evident at the time of the complete eruption. The degree of the leucocytosis corresponds as a rule with the severity of the disease and in some cases is diminished at the time of the eruption, but usually continues for several days and may even extend for weeks after the temperature has subsided.

The increase in the number of leucocytes is largely referable to the polynuclear cells, these constituting from 85 to 99 per cent. of the total number. The lymphocytes diminish in the early stages of the disease, but later rise to normal or slightly above normal figures. The eosinophile cells are usually normal or even subnormal at first, but steadily increase as the disease progresses and reach a degree of 10 to 20 per cent. in the second or third weeks, after which they slowly decline. These rules are not invariable in scarlet fever, but a severe leucocytosis appearing prior to the period of eruption of an infectious fever is practically always suggestive of this disease. In some cases the polynuclear cells diminish about the end of the first week and the lymphocytes and eosinophiles rapidly increase, leading to a later secondary leucocytosis.¹

(4) Measles.

This condition shows in itself nothing particularly characteristic in the blood, but the absence of definite findings is of great importance in its differentiation from scarlet fever with which it might be confounded. The red cells in this disease are not found to be greatly changed, although a slight reduction in their number is usual. A loss of hemoglobin is practically always noticed.

The leucocytes are usually normal or slightly reduced in number at the outset of the disease, being the lowest at the height of the eruption when the figure may reach as low as 2,500 cells, returning to the normal within a few days after subsidence of fever. A complicating bronchitis may cause a moderate leucocytosis of 8,000 to 16,000 cells, but this should not lead one to a mistaken diagnosis, as the clinical symptoms of both scarlet fever and of

¹ Döhle (Centralbl. f. Bakteriöl., 1911, LXI, 63; Ibid., 1912, LXV, 57; Münch. med. Wchnschr., 1912, LIX, 1688) has announced the almost constant presence in the polymorphonuclear leucocytes of "inclusion bodies." These are observed near the margin of the cell as rod or coccus forms, which stain readily a faint bluish-green tint with the Giemsa or other blood stains. While these bodies are observed in about 95 per cent. of scarlet fever cases in the early days of the disease, the number diminishes gradually. They are also found in many cases of diphtheria, pneumonia and tuberculosis, yet they are very infrequent in cases of serum sickness or of scarlatiniform rashes. Their presence is, therefore, not pathognomonic but their absence almost excludes scarlet fever. See Kretschmer, Berl. klin. Wchnschr., 1912, XLIX, 499; Deutsch. med. Wchnschr., 1912, XXXVIII, 2163; Nicoll and Williams, Arch. Pediat., 1912, XXIV, 350; Ahmed, Berl. klin. Wchnschr., 1912, XLIX, 1232; Kolmer, Am. Jour. Dis. Child., 1912, IV, 1; Granger and Pole, Brit. Jour. Child. Dis., 1913, X, 9; Lippmann and Hufschmidt, Zentralbl. f. inn. Med., 1913, XXXIV, 369; Schippers and de Lange, Berl. klin. Wchnschr., 1913, L, 544; Bongartz, Ibid., 544; Schwenke, Münch. med. Wchnschr., 1913, LX, 752; Pappenheim, Folia Hæmat., 1913, XV, 379; Cummins, Jour. Med. Research, 1913, XXVII, 529; Nicoll, Arch. Pediat., 1913, XXX, 346; and Brinckmann, Berl. klin. Wchnschr., 1913, L, 1248; Massini, Med. Klin., 1913, IX, 1729; MacEwen, Jour. Path. and Bacteriol., 1914, XVIII, 456; Rosanoff, Arch. f. Kinderhke., 1914, LXII, 321; Hill, Boston Med. and Surg. Jour., 1914, CLXX, 792; Isenschmid and Schemensky, Münch. med. Wchnschr., 1914, LXI, 1997; Kalser and Löwy, Deutsch. Arch. f. klin. Med., 1914, CXVI, 82; Rehder, Ibid., 1914, CXVII, 37; Accoyer, Presse Méd., 1922, XXX, 401.

measles should be well-established at the time of the complicating bronchitis. An eruptive fever in the second or third day of its course should be considered scarlet fever, or at least scarlatina, if a leucocytosis is present, while if the disease be measles the number of leucocytes will be normal or even subnormal in the absence of extensive bronchitis.¹

(5) Variola.

This condition is associated with more or less extensive destruction of the red cells.² In the early stages of the disease the red count may be slightly above normal, owing to the concentrating effect of the fever, but later the red cells will show a sudden reduction. This reduction is especially noticeable in the cases associated with extensive pustulation, when the septic process has such a marked influence in destroying the red cells. The hemoglobin is usually reduced in degree parallel to the diminution in red cells.

In most of the cases of small-pox we find a distinct leucocytosis which may run from 10,000 to 20,000 as a rule, but has reached as high as 41,000 in the severe cases. This leucocytosis begins with the appearance of the vesicle, increases as the exudate becomes purulent, and reaches its height when suppuration becomes extensive; that is, the degree of leucocytosis usually runs parallel to the severity of the septic process, the count returning gradually to normal as the suppuration subsides. The leucocytosis in small-pox is usually of the lymphocytic type, the number of these cells varying from 35 to 45 per cent. of the white cells. Associated with the increased lymphocyte value we find from 5 to 10 per cent. of the large mononuclear leucocytes and usually an average of 3 per cent. of neutrophile myelocytes, in some cases these latter running as high as 16 per cent. Eosinophiles and basophiles are occasionally observed, especially in the hemorrhagic form of this disease.

It will thus be seen that small-pox causes a leucocytosis which may reach even the degrees given by scarlet fever, but the differential count as shown by the large percentage of lymphocytes in the former and the greatly increased number of polynuclear neutrophiles in the latter should make a mistake in diagnosis impossible. It is to be remembered that complications of true abscesses with the pustules of small-pox may increase the percentage of polynuclear cells in this disease, but never to such a degree as is shown in scarlet fever.

An examination of the blood of children, who have been vaccinated with small-pox virus, shows a distinct leucocytosis of the polynuclear type, reaching as high as 20,000 cells. This leucocytosis usually begins on the third or fourth day after inoculation and gradually subsides until the end of the period of vaccination.

¹ Schwaer (Münch. med. Wchnschr., 1913, LX, 1203) believes that a diminution or disappearance of eosinophiles at the height of the fever is characteristic of measles. See, also, Lucas, Am. Jour. Dis. Child., 1914, VII, 149; and Grumann, Münch. med. Wchnschr., 1914, LXI, 132. Hess (Arch. Int. Med., 1914, XIII, 913) discusses the blood picture in German measles.

² See Proescher (Interstate Med. Jour., 1915, XXII, 427) for a discussion of the cultivation of the variola-vaccine virus.

The blood in cases of varicella¹ seems to show the same characteristics as that of very mild cases of variola, or of vaccinia. The slight leucocytosis is usually of the polynuclear type, reaching a degree of 15,000. The large mononuclear cells which seem to play such an important rôle in the differential leucocyte picture of variola are for the most part absent in varicella, and myelocytes are practically never found in this condition.

(6) Diphtheria.

The number of red cells in diphtheria seems to be slightly increased, owing to the marked concentration of the blood in this condition. In practically all cases of diphtheria the number ranges from a high normal value to as high as 7,800,000 reported by Cuffer. As the disease progresses, especially after the temperature has fallen, the number of red cells is diminished and a coincident decrease in the percentage of hemoglobin is observed. This slight anemia is not evident in the early stages of the disease due, no doubt, to the abnormal concentration of the blood.

In this condition, like most infections, we find a leucocytosis ranging between 25,000 and 50,000, the higher the leucocytosis the more grave the prognosis. In one case reported by Felsenthal the leucocytes numbered 148,000, but this is very unusual, as the grade of leucocytosis is ordinarily proportional to the extent and depth of the membrane. The leucocytosis is usually of the polynuclear type, the lymphocytes being also slightly increased. In some cases a lymphocytosis of 60 per cent. (Ewing) has been observed, but this is not the usual finding. The eosinophile cells are reduced in number, but are relatively more numerous than in pneumonia. It is in diphtheria that we find quite marked degenerative changes in the leucocytes, "the leucocyte shadows" and increased acidophile tendency of the neutrophile granules being especially worthy of mention.

(7) Pertussis.

The exact work of Barach² and others has so modified our ideas of the changes in the blood in pertussis that I can do no better than to give his summary upon these points:

"In the early stages of this disease there is a leucocytosis with increase of all the forms; then a small-cell lymphocytosis becomes conspicuous and continues to increase when the other forms have reached their limit. The large lymphocytes follow the course of the small ones, but they reach their greatest numbers after the small cells have reached theirs. During the stage of active lymphocytosis, bilobed, small lymphocytes are frequently seen as well as numerous degenerated large lymphocytes, especially the basket forms. Then comes the simultaneous falling of the leucocytosis and lymphocytosis, while the polynuclears begin to resume their normal proportion. A little later the mast cells are observed more frequently, and an occasional myelocyte

¹ See Mensi, *Gazz. d. Osp.*, 1912, XXXIII, 1625; also, Erlenmeyer, *Deutsch. med. Wchnschr.*, 1913, XXXIX, 21; Schatzmann, *Cor.-Bl. f. Schweiz. Aerzte*, 1913, XLIII, 1515; *Ztschr. f. klin. Med.*, 1914, LXXX, 333; Erlenmeyer and Jalkowski, *Deutsch. med. Wchnschr.*, 1914, XL, 646; Force, *Jour. Lab. and Clin. Med.*, 1916, I, 243.

² *Arch. Int. Med.*, 1908, I, 602. See, also, Schneider, *Münch. med. Wchnschr.*, 1914, I, XI, 303; Hess, *Ztschr. f. Kinderhkte.* 1920, XXVII, 117; Bourne and Scott (*Brit. Med. Jour.*, 1922, I, 387) report a case of pertussis showing a leucocytosis of 176,000.

may be seen. While the leucocytosis and lymphocytosis continue to fall by lysis, an eosinophilia is noted; this continues for a variable time, after which the blood formula resumes its normal proportions. During this entire cycle the transitionals seem unaffected.

If we were to speak of the first and second half of the blood cycle in this disease, we would say that in the first half the lymphocytes are the prominent factors and in the second half the polynuclears and the eosinophiles.

Clinically, leucocytosis is present at about the time the child first coughs; as the coughing goes on, the leucocytosis increases and the lymphocytosis becomes very marked. Churchill believes that a lymphocytosis exists in the prespasmodic stage and is of extreme importance in early diagnosis. The height of the leucocytosis is reached in the spasmodic stage, sometimes early, and sometimes in the latter part, the sickest children showing the highest grade of leucocytosis. About the time that a marked improvement is noted in the child the leucocytosis has decreased, the polynuclears have increased and the eosinophilia is present."

The degree of leucocytosis in this condition varies between 25,000 and 51,150.

(8) **Acute Rheumatism.**

In this disease we find that the red cells are quite markedly destroyed, causing, very frequently, a reduction of 2,500,000 cells. This reduction is not always evident in the early stages of the disease, as the blood becomes very much concentrated by the marked sweating which is such a prominent symptom of the disease. The hemoglobin suffers more than the red cells so that we may find the percentage of hemoglobin as low as 60 per cent. in this condition. This anemia is one of the characteristic signs of acute rheumatism and continues well on into convalescence, the hemoglobin not being as quickly restored as are the red cells. A largely increased formation of fibrin has been observed and may have some diagnostic importance.

The leucocytes are increased in proportion to the severity and acuteness of the disease, the grade being usually moderate and the type being polymorphonuclear. In the very mild cases we may find no leucocytosis and one reaching 20,000 or more is, according to Türk, always associated with complications. As the fever diminishes the leucocytes return to normal and are not as much affected by the subsequent attack as by the previous initial one. The eosinophile cells are absent only in the early stages, while they are present in moderate amounts later in the disease and show a distinct increase after defervescence (Loeffler).

It would be impossible in a work of this character to discuss in detail the blood changes in all diseases, whether infectious or non-infectious.¹ The writer, therefore, has selected under the acute infectious diseases those which show the more characteristic changes in the blood and those in which an examination of the blood is more frequently called upon to aid in diagnosis. Many phases of these diseases have been left to the chapters on bacteriology of the blood.

¹ See Barach (*Arch. Int. Med.*, 1913, XII, 751) for blood changes in mumps. Hess and Fish (*Am. Jour. Dis. Child.*, 1914, VIII, 385) discuss the blood in infantile scurvy.

(d) Blood in Chronic Infections.

(1) Tuberculosis.

The earlier studies of the blood of tubercular patients reveal the fact that the blood may show practically no changes which are comparable with the pallor of the skin and the degree of the emaciation of the subjects affected. An anemia is often seen of the very highest type, but usually one of moderate degree is present and even may not exist at all. The degree of anemia is independent of the localization of the disease, although pulmonary affections are more frequently associated with high-grade anemia than are other tubercular conditions. It is to be remembered that pulmonary tuberculosis is so frequently associated with extensive hemorrhage that one may not wonder at the severe anemia present, yet we find cases in which the regeneration is very rapid after severe hemoptysis. As a rule, a mild anemia of the chlorotic type prevails; that is, the count is practically normal with the hemoglobin somewhat reduced. Occasionally we find a slight lymphocytosis, especially of the smaller cells, and only when a secondary infection prevails does the leucocytosis take on the polynuclear type.¹ The lymphocytosis in tuberculosis is so common that we usually find even in the sputum an excess of the small mononuclear cells in the pure tubercular affection of the lungs.

In tubercular infection of the meninges we practically always find a leucocytosis, but with this exception uncomplicated tuberculosis is not associated with an increase in the number of white cells. It is highly probable that the increased percentage of the mononuclear cells is more closely associated with the poor nutrition which the tubercular patient shows than it is with any specific effect of the bacillus tuberculosis. Occasionally we find the eosinophile cells somewhat increased, especially in pulmonary conditions with cavity formation, but it must be remembered that a slight eosinophilia will obtain if tuberculin therapy is being used in such cases.

(2) Syphilis.

According to Becquerel and Rodier, a moderate grade of anemia is to be found in the majority of cases of syphilis, becoming more pronounced as the disease progresses. This anemia of syphilis is of the chlorotic type, but may increase until the pernicious type becomes established. As a rule, the reduction in the number of red cells is moderate, being rarely below 3,000,000 cells. The hemoglobin is usually relatively more decreased than are the cells, and the application of mercury in the treatment of this condition frequently lowers this percentage still further, establishing an anemia which is directly referable to the mercury. This reduction in the number of red cells and in hemoglobin becomes more marked as secondary symptoms appear, so that a diagnosis of an initial lesion becomes established by a later examination of the blood.

The increase in the number of leucocytes is largely limited to the secondary and tertiary stages of this disease, as the leucocytes usually remain normal

¹ See Miller and Reid, *Arch. Int. Med.*, 1912, IX, 609; Miller, Lupton and Brown, *Am. Jour. Med. Sc.*, 1912, CXLIII, 683; Ringer, *Ibid.*, 1912, CXLIV, 561; also, Rayevsky, *New York Med. Jour.*, 1913, XCVII, 813; Wack, *Deutsch. Arch. f. klin. Med.*, 1914, CXV, 596; Morgan, *Am. Jour. Dis. Child.*, 1916, XI, 224.

up to the time of the eruptive stage. The increase in the secondary stage, which may reach as high as 20,000, is largely in the number of the small and large lymphocytes, but the eosinophile cells may be increased to as high as 5 per cent. in some cases. In very severe cases a progressive polynuclear leucocytosis is observed. As the tertiary stage comes on the leucocytosis usually persists, but the lymphocytosis becomes less distinct and constant through the increase in the number of polynuclear cells.¹

Justus' Test.

Justus has found, in studying the blood of patients suffering with florid syphilis, that injection or inunction of preparations of mercury cause a reduction in the percentage of hemoglobin of from 10 to 20 per cent. for a period of a few hours or days. After a certain time, varying with the general condition of the patient and the severity of the symptoms, the hemoglobin increases again. This test can hardly be considered diagnostic of syphilis, as the mercury salts all cause an anemia which may be directly traceable to their hemolytic action upon the red cells.

In the blood of patients suffering with congenital syphilis we always find a distinct anemia, associated with a slight leucocytosis, especially of the lymphocytic type. The red cells in this condition show many changes, such as polychromatophilia and nucleation, while the changes in the white cells often resemble the picture of a mild grade of leukemia. For a discussion of the causative factor, the spirochæta pallida, see the section on Parasitology (p. 676).

(3) Leprosy.

The blood in leprosy is quite different from that in either of the two previous conditions. The usual rule is a very slight reduction in the number of red cells, although cases have been reported with a red count of 1,900,000 and a blood picture of pernicious anemia. The hemoglobin does not seem to be reduced to any extent, the percentage usually being relatively higher than the number of red cells, so that a high color index almost invariably obtains. The leucocytes are rarely increased in number, being usually subnormal, with a relative increase in the number of lymphocytes, their percentage reaching as high as 47 per cent., according to Winiarski.

(4) Carcinoma.

Although discussed under the heading of Chronic Infections, carcinoma has at present no etiological relation to such conditions. This is one of the most important causes of anemia, owing to the frequent hemorrhages and the mechanical effects of the growth as well as to the unknown toxin, which may produce severe constitutional symptoms, even though the growth may have become latent. The anemia of malignant disease usually runs parallel to the progressive cachexia. The grade of anemia may vary, depending upon the location of the tumor, from a very mild chlorotic anemia to one with a perfect picture of pernicious anemia. It is natural to suppose that the more malignant the disease the greater will the blood changes be, so that we should expect to find the rapidly growing cancers which form numerous metastases usually associated with the more extreme blood picture. That this is not

¹ See Hazen, *Jour. Cutan. Dis.*, 1913, XXXI, 618 and 739.

always the case is shown by the following statement of Emerson: "Our cases with rapidly developing metastases, with large nodules, are those with a slight chlorotic anemia; those which simulate pernicious anemia are more often those with few objective signs of cancer, an insignificant-looking little nodule." It is possible that this paradox may be explained by the fact that the development of the cancer is so rapid that the toxin has not had sufficient time to cause the blood changes which the more slowly developing growth may bring about. The changes in metabolism are much more marked in the slowly developing cancers than in the more rapidly growing ones, so that we might assume that the same rule applies to the blood changes as expressions of the general systemic disturbances.

When the anemia of cancer develops it is usually more severe than in any other chronic disease. The chief changes are at first in the size, shape, weight, and degeneration of the red blood-cells; later, as the cachexia develops the red cells are often as low as 2,500,000 or even as low as in pernicious anemia, 1,000,000. The hemoglobin is always reduced in amount, but is rarely as low as in chlorosis, the average, according to Cabot, being about 58 per cent. The hemoglobin value seems to be lower in cases of visceral cancer than in those of peripheral type. In a majority of the cases a moderate leucocytosis obtains which is never seen in the benign tumors unless these be complicated by supuration. This leucocytosis depends largely upon the amount of hemorrhage from the tumor and upon the position of the cancer. We find in carcinoma of the stomach and uterus, in which hemorrhages are very frequent, quite an extensive leucocytosis, while in cancer of the esophagus a leucopenia may obtain. The larger and faster the tumor grows the greater will be the degree of leucocytosis, a condition which is the reverse of that usually found in the case of the red cells. The leucocytosis of cancer is usually of the polynuclear type, but this may not be over 45 per cent., in which cases the lymphocytes are relatively increased. The eosinophiles are rarely as much diminished as in other conditions, but they are not always increased. Myelocytes are, perhaps, more frequently found in cancer than in the other types of anemia, excepting pernicious anemia and leukemia.¹

The degree of cachexia is often very extensive in cancer, but is not always so closely related as one would suppose to the changes in the blood. In those cases in which the cachexia is due to a combination of malnutrition with intoxication by the malignant toxin the blood changes are naturally very severe, but, as previously stated, the more fulminating types of cancer are not associated either with great cachexia or with severe changes in the blood. Cachexia, therefore, seems to be more a function of the chronicity of cancer than of its malignancy.

The changes in the blood in the numerous specific diseases of various organs show nothing characteristic in themselves. It is to be expected that in all chronic diseases of whatever organ, a slight anemia may be present owing to the effects of such disorders upon the general metabolism. These changes are, however, of the general type of simple anemia and are usually

¹ See von Roznowski, *Ztschr. f. klin. Med.*, 1915, LXXXI, 377.

rapidly remedied by the application of the ordinary therapeutic agents. It is true that disease of the general organs causes changes in the composition of the fluid portions of the blood, which may bring about a secondary change in the cellular content.

(e) Effects of Splenectomy.

The effects of splenectomy are usually the combined results of severe hemorrhage, a preexisting anemia, of the loss of functions of this organ, and of intravenous infusion¹ which has been performed following the operation (Ewing). In comparatively healthy subjects splenectomy has often been performed without affecting the blood more than does any other abdominal operation. Immediately after removal of the spleen an increase in the blood pigment and a polycythemia occur (which are probably due to diminished hemolysis in the absence of the spleen) providing there is an adequate supply of iron in the diet. The most marked changes in the blood are seen in those cases in which the organ has been removed for rupture or idiopathic enlargement, the loss of blood and the shock of operation giving rise to a considerable degree of secondary anemia.

The red cells are frequently restored to normal in one to three months, due to hyper-activity of the bone-marrow, but in some cases which progress less favorably the anemia may be more persistent. The restoration of hemoglobin does not take place, as a rule, as rapidly as does that of the red cells. Following the operation we usually observe a polynuclear leucocytosis which may run, as in one case observed by the writer, as high as 75,000 cells. This leucocytosis usually lasts from one to two months, but may persist for several months, in which case the polynuclear cells are replaced by lymphocytes. Eosinophilia usually develops early and has been observed in some cases two or three years after operation. In some cases, especially those suffering from extensive hemorrhage, a very profound anemia characterized by great diminution in the number of red cells, the presence of polychromatophilic and degenerated cells, nucleated red cells, and a high degree of leucocytosis is observed. The leucocytes in these cases may take on the picture of an acute leukemia, but this condition is transitory as the blood improves more or less rapidly. A leucocytosis or permanent lymphocytosis are probably the only specific effects of splenectomy.²

V. PARASITOLOGY OF THE BLOOD

(1) Malaria (Paludism; Hemamebiasis).

Malaria is a disease caused by the entrance of an animal parasite into the

¹ See Ottenberg and Kaliski, *Jour. Am. Med. Assn.*, 1913, LXI, 2138; Nobel and Steinebach, *Ztschr. f. Kinderhke.*, 1914, XII, 75.

² See Musser, *Arch. Int. Med.*, 1912, IX, 592; Giffin, *Am. Jour. Med. Sc.*, 1913, CXLV, 781; Sollenberger, *Biochem. Ztschr.*, 1913, LV, 13; Musser and Krumbhaar, *Jour. Exper. Med.*, 1913, XVIII, 487; Pearce and Pepper, *Jour. Exper. Med.*, 1914, XX, 19; Krumbhaar and Musser, *Ibid.*, 108; Austin and Pearce, *Ibid.*, 122; King, *Arch. Int. Med.*, 1914, XIV, 145; Morris, *Ibid.*, 1915, XV, 514; Pearce, Austin and Pepper, *Jour. Exper. Med.*, 1915, XXII, 682; Sellards and Minot, *Jour. Med. Res.*, 1916, XXXIV, 460; Mayo, *Jour. A. M. A.*, 1916, LXVI, 716; Lee, Minot and Vincent, *Ibid.*, LXVII, 719; Krumbhaar, *Ibid.*, 723; Miller, *Ibid.*, 727; Friedman and Katz, *Ibid.*, 1205; Schneider, *Arch. Int. Med.*, 1916, XVII, 32; Pepper and Austin, *Ibid.*, XVIII, 131; Schneider, *Ibid.*, 1917, XIX, 156; Orr, *Jour. Lab. and Clin. Med.*, 1917, II, 895; Hall, *Am. Jour. Med. Sc.*, 1920, CLX, 72.

blood and its development within the red blood-corpuscle. This parasite was first studied by Laveran and belongs to the class of sporozoa. It was not until the recent work, especially of Grassi, Ross, and Nuttall, that we were enlightened as to the source of this invader. It is at present well established that the malarial parasite runs its sexual cycle (*sporogone*) within the body of the anopheles mosquito (*Anopheles maculipennis*).¹ The old idea that malaria was an air-borne disease, the contagion arising from stagnant pools in swampy regions must now be replaced by the modern mosquito theory. It is true that the anopheles lays its eggs upon the surface of almost stagnant water and that the larvæ hatch in these places. The eggs are boat-like in shape (each separate, the groups being arranged in ribbons) and float upon the surface, while the larvæ lie just below the surface and are in a plane parallel with it. These facts have led to the adoption of the modern methods of prevention of malaria by covering the surface of such stagnant pools with oil, which prevents access of air to the larvæ and, in consequence, causes death.²

In a discussion of malaria it must be remembered that there are several types of this disease, depending on (1) the kind of parasite causing the infection and (2) the period at which the various groups of the same parasite run their asexual course in the host. We have, therefore, to discuss the three types of infecting organism, each of which is a protozoan form and is found in the red cells. The cycle of development of the tertian organism is approximately 48 hours, so that with a single infection paroxysms will occur on alternate days. With the quartan organism the cycle of development requires 72 hours, while with the estivo-autumnal form it is variable, running from 24 to 72 hours. It is, of course, possible to have infection with more than one form of parasite or with more than one series of the same parasite, so that we may have daily exacerbations through infection with any one of these three types of parasite.

In an examination of the blood for the malarial parasite a study of the fresh specimen is always desirable when possible, as the peculiar ameboid movements of the parasite as well as the rapid oscillatory movements of its granules can, of course, not be seen in the fixed specimen. Moreover, the peculiar brassy tone of the red cell and the irregularity in shape and size of these cells may best be studied in the fresh specimen. The beginner, however,

¹ Different species of the anophelinæ vary in their ability to transmit malaria. While more than 100 species are known, many have not been studied. See Walker and Barber, Phil. Jour. Sc., B, 1914, IX, 381; also, Ludlow, Disease-bearing Mosquitoes, Bull. 4, War Dept. Office of the Surgeon General, 1914. Recent work has established the fact that *Anopheles punctipennis* is an efficient host of the organisms of tertian and estivo-autumnal malaria; anopheles crucians of estivo-autumnal type; and anopheles quadrimaculatus of the tertian and estivo-autumnal forms. See King, Science, 1915, XLII, 873; Am. Jour. Trop. Med. and Prev. Med., 1916, III, 426; Jour. Exper. Med., 1916, XXIII, 703; Mitzmain, Public Health Reports, 1916, XI, 301. Darling (Jour. Exp. Med., 1920, XXXII, 313) reports the successful infection of the *Anopheles ludlowi* with the aestivo-autumnal parasite and its transmission to man, while Hylkema (Mendedeel. v. d. Burg. Geneesk., 1920, p. 50) succeeded in infecting 20 to 50 per cent. of this same mosquito with the quartan organism Blacklock and Carter (Ann. Trop. Med. & Parasitol., 1920, XIII, 413 and 421) have been able to infect laboratory bred *Anopheles plumbeus* and *Anopheles bifurcatus* with the tertian parasite.

² See McCoy, Pub. Health Rep., 1912, XXVII, 1029; also Craig, The Prophylaxis of Malaria, Bull. 6, War Dept., Office of Surgeon General, 1914; von Ezdorf, Pub. Health Rep., 1913, XXVIII, 2830; Ibid., 1914, XXIX, 503, 613, 871, 1073 and 1289.

will find that a stained specimen will yield much more definite results, providing his staining technic is good than will a study of the fresh specimen, as the slight refractility both of the cell and of the parasite in the fresh specimen makes it difficult in every case to get the proper illumination of the specimen. In the hands of an expert the examination of fresh blood is practically all that is required for a diagnosis in the average case, and when the parasites are moderately numerous the beginner can scarcely make a mistake. It would seem, therefore, inadvisable to rest a diagnosis upon an examination of the fresh specimen in cases in which no organisms are found, but to control this examination by a careful study of a stained specimen in which one may frequently be surprised at the number of parasites to be seen, although a negative result has been observed in the unstained specimen. A word of caution, however, is necessary at this point. Frequently one observes in stained specimens many artefacts due to deposition of staining pigments upon the red cell, while in the fresh specimen areas of coagulation necrosis are not infrequently seen, so that the untrained observer may assume the presence of malarial organisms. For an absolute diagnosis of malaria it is necessary to find intracellular organisms, and not to be content with a single examination in doubtful cases.¹

Examination of Fresh Blood.

The technic of making a fresh specimen of suspected blood is the same as that previously outlined and consists in touching a perfectly clean cover-slip to a drop of blood and allowing the cover-slip to fall upon a clean glass slide. The quantity of blood should be rather small so that the red cells may be distinctly separated from one another. The examination is best made by the use of a $\frac{1}{2}$ immersion lens.

(a) The Tertian Organism (*Hemameba vivax*; *Plasmodium vivax*).

The youngest form of the tertian parasite as it appears in the red cell resembles very closely the spore of the parent rosette.² It is a small, compact,

¹ Bass has recently succeeded in cultivating the various types of malarial plasmodia., See Bass, Jour. Am. Med. Assn., 1911, LVII, 1534; Bass and Johns, Jour. Exper. Med., 1912, XVI, 567; Lavinder, Jour. Am. Med. Assn., 1913, LX, 42. The parasites are cultivated only in the red cells of human blood and are destroyed by the leucocytes as well as by the serum. See, also, Sinton, Ann. Trop. Med. and Parasitol., 1912, VI, 371; and Olpp. Münch. med. Wchnschr., 1912, LIX, 2623. Thomson and Thomson (Proc. Roy. Soc. London, 1913, LXXXVII, 77, and Ann. Trop. Med. and Parasitol., 1913, VII, 153) show that cultures of benign tertian parasites differ from those of the malignant tertian forms in that there is no tendency to clumping of the former types. This may explain why we do not find any but the young forms of the malignant tertian organism in the peripheral blood, as the clumping causes the larger older forms to collect in the internal organs. Ziemann, Centralbl. f. Bakteriöl., 1. Abt., Orig., 1913, LXVII, 482; Sergent, Beguet and Plantier, Compt. rend. Soc. de biol., 1913, LXXV, 324; Bass, Am. Jour. Trop. Dis. and Prev. Med., 1914, I, 539. Miller (Jour. Am. Med. Assn., 1914, LXII, 1549) reports the cultivation and development of a second generation of parasites without the removal of the leucocytes. See, also, Clarke, Lancet, 1917, I, 530.

² Stephens (Proc. Roy. Soc. London (B), 1914, LXXXVII, 375, and Ann. Trop. Med. and Parasitol., 1914, VIII, 119) reports the discovery of a new malarial parasite, which he names *Plasmodium tenue*. Craig (Jour. Parasitol., 1914, I, 85) believes this to be nothing more than a rather atypical form of *Plasmodium vivax* in the unpigmented stage of development. Lawson (Jour. Exper. Med., 1916, XXIV, 291) regards the *plasmodium tenue* as not a new type but a parasite attached to the external surface of red cells and distorted by technic.

Emin (Bull. Soc. Path. Exot., 1914, VII, 385) describes a further variety of the tertian organism to which he gives the name *Plasmodium vivax* variety *minuta*. Craig does not believe the evidence sufficient to give this parasite specific rank.

PLATE XXVIII.



THE TERTIAN PARASITE.

1. *Normal erythrocyte.*
- 2, 3, 4, 5. *Intracellular hyaline forms.*
- 6, 7. *Young pigmented intracellular forms.* In 6 two distinct parasites inhabit the erythrocyte, the larger one being actively ameboid, as evidenced by the long tentacular process trailing from the main body of the organism. This ameboid tendency is still better illustrated in 7, by the ribbon-like design formed by the parasite. Note the delicacy of the pigment granules, and their tendency toward peripheral arrangement in 6, 7, and 8.
8. *Later developmental stage of 7.* In 7, 8, and 9 enlargement and pallor of the infected erythrocyte become conspicuous.
9. *Mature intracellular pigmented parasite.*
- 10, 11, 12. *Segmenting forms.* In 10 is shown the early stage of sporulation—the development of radial striations and peripheral indentations coincidentally with the swarming of the pigment toward the center of the parasite. The completion of this process is illustrated by 11 and 12.
13. *Large swollen extracellular form.* Note the coarse fused blocks of pigment. (Compare size with that of normal erythrocyte, 1.)
14. *Flagellate form.*
15. *Shrunken and fragmenting extracellular forms.*
16. *Vacuolation of an extracellular form.*

NOTE.—The original water-color drawings were made from fresh blood specimens, a Leitz $\frac{1}{2}$ -inch oil-immersion objective and 4 ocular, with a Zeiss camera-lucida, being used.

(E. F. FABER, *sec.*)

(From Da Costa's "Clinical Hematology.")

colorless, non-pigmented disk (hyaline form) about 2 microns in diameter and shows an undulating outer rim of basophilic protoplasm which encloses a single large nuclear body which does not stain with methylene blue but shows a distinct chromatin stain with any of the modifications of the Romanowsky stain. This nuclear body is usually surrounded by a clear space which does not take the stain and which has been termed by Gautier "*the milky zone.*" The parasite has a very rapid ameboid movement and shows a great number of changes in shape and position. It sometimes assumes a typical ring-like form which is usually a little thicker at one point, from which the name "*signet ring*" has been given. Occasionally several of these rings may be seen within a single blood-cell. After about 12 hours the corpuscle increases slightly in size, becomes somewhat paler, but still has the sharp, smooth, round outline of the normal cell. At this stage the ameboid powers of the organism are very marked, so that many pseudopodia may be seen, connected to the larger part of the organism by very thread-like pale and rather indistinct bands of union. This gives the appearance of disconnected globules of protoplasm, which is very slightly refractile. At this period (12 hours) pigment (hematin) appears in the parasite in the form of very fine light brown granules which have a very rapid dancing motion and are clustered especially at the ends of the pseudopodia. The organism continues to increase in size and, at the same time, the host becomes somewhat larger, paler, but still round in outline. At the end of 24 hours the organism fills about one-third of the cell, is still ameboid and shows increased pigment, which is somewhat darker in color and is less actively motile, being distributed throughout the substance of the parasite. In this form one may occasionally see the nucleus as a globular body at the end of a pseudopod. In the last half of the cycle of development of the tertian organism the growth is much more rapid than in the first half, the parasite being fully developed within 40 hours. The cell at this time is about one and a half times its normal size and is so little refractile that its outline can scarcely be seen. The organism is from 8 to 10 microns in diameter, is round, and is even less refractile than is the corpuscle. The pigment is much more abundant at this time and is still evenly distributed throughout the organism. The next stage in the development of the organism is known as the *presegmenter stage*. The cell becomes practically invisible, the pigment collects in one or more irregular clumps throughout the organism, the granules moving in irregular lines. At this time the periphery of the organism shows slight crenation and refractive dots appear irregularly in the periphery of the organism. The line of demarcation between the presegmenter and the segmenter is very slight. The corpuscle is now practically eliminated and the organism becomes more dense and highly refractile. The refractive dots which were visible in the presegmenter stage are now seen to be in the center of lines of separation which pass from the irregular crenated border down toward the center of the organism, thus marking off future segments, which are as a rule from 15 to 20 in number. As development proceeds the segments become more sharply defined until the clumps form into discrete circular masses with a distinctly refractile spot in the center. The pigment in these segment forms

seems to be left in masses between the segments without any definite arrangement. Each segment now splits off from the mother segmenter cell and becomes free in the blood in the form of the original hyaline type which becomes attached to a red cell and soon enters it to pass through the various stages discussed. It is to be remembered that the hyaline forms do not modify their host, either in shape, color, or size, such changes being observed only at the time when the pigment first becomes evident.

The preceding is a concise description of the cycle of development within the cell (*asexual generation or schizogone*), but we occasionally find tertian forms other than hyaline which are extracellular. These seem to be of two types, the degeneration forms and the *gametocytes*, cells capable of sexual development. The degeneration forms or, as they are sometimes called, extruded intracellulars, are sometimes the only ones seen in the specimen. These are parasites which have passed from the cell and have died, the organism sometimes appearing as if it had passed out through a very fine hole. If it be entirely extruded, the hemoglobin leaves the cell with it and only a shadow of the red corpuscle remains behind. However, this does not always occur, so that we meet with typical dumb-bell-shaped organisms in the plasma. If the blood be observed while this process of extrusion is going on, the pigment will still be extremely active but it gradually becomes quiet as the organism dies. The organism may break up into fragments forming several pigmented spherical masses or it may become deformed and vacuolated, constituting the so-called "sporulating" forms. The gametocytes are found at all times in the blood after the infection has been established for a few days. These are not in reality extracellular forms as one sees them in the stained specimens surrounded by the shell of the corpuscle. These gametocytes are of two forms, the *macrogamete* or female cell and the *microgamete*, the male cell, which is one flagellum of the *microgametocyte* (parent male cell). The macrogametes are large organisms, pale, indistinct, and three or four times as large as the red cell. Some of them show no trace of the corpuscle; their pigment is abundant and exhibits very active movements. Their nucleus is about three and one-half microns in diameter and is sometimes seen in the fresh specimen; either its outline is distinct or its size and shape may be recognized, as it is the only portion of the parasite which is not invaded by the pigment granules. The function of these macrogametes seems to be to continue the life of the organism within the mosquito after the organism has become fertilized by the male element or microgamete. The microgametocyte is smaller than the macrogamete, being eight to ten microns in diameter. Its pigment is very active, but soon forms a circle around the center and becomes stationary. Occasionally this pigment may become even more active than before, the margin of the cell may undulate and several flagella protrude. These flagella are the microgametes and are two or three times the length of a red blood-cell and often contain pigment granules which enable them to be followed when they break loose from the parent cell. After these flagella separate, the parent cell is seen as a small cell with central motionless pigment. This process of flagellation is not seen in the fresh specimen, but occurs 15 or

PLATE XXIX.



THE QUARTAN PARASITE.

1. *Normal erythrocyte.*
2. *Intracellular hyaline form.*
3. *Young pigmented intracellular form.* Note the coarseness, dark color, and scantiness of the pigment granules.
- 4, 5, 6, 7. *Later developmental stages of 3.* Note the peripheral distribution of the pigment in all the parasites from 3 to 8. (Compare size and color of the erythrocytes in 5, 6, and 7 with 7, 8, and 9, Plate VI.)
8. *Mature intracellular form.* Note that the stroma of the erythrocyte is no longer demonstrable.
- 9, 10, 11. *Segmenting forms.* In 9 are shown the characteristic radiating lines of pigment. (Compare with 10, 11, and 12, Plate VI, and with 10, 11, and 12, Plate VIII.)
12. *Large swollen extracellular form.* (Compare with 13, Plate VI.)
13. *Placellate form.* (Compare with 14, Plate VI.)
14. *Vacuolation of an extracellular form.*

(E. F. FABER, *sec.*)

(From Da Costa's "Clinical Hematology.")

20 minutes after the blood has been drawn, which would point to the fact that such a process does not occur within the body. Normally, this change takes place only in the stomach of the mosquito.

(b) **The Quartan Organism (*Hemamebamaris*; *Plasmodium malaris*)**

This organism is much more rare than is the tertian. Its cycle of development requires 72 hours so that the normal paroxysm occurs every fourth day. If two groups are causing the infection there will be two days with paroxysms, one free day, and then two days of paroxysm following. If more than two groups are introduced we may have daily chills and fever, but only when the groups are large enough in number to cause a paroxysm. According to Ross, 250,000,000 organisms are necessary before a chill follows. The small hyaline forms of the quartan parasite are not distinguishable in the early stages from those of the tertian type, but are easily recognized at the time pigment appears, as the granules of the former are coarser, darker in color, and not so actively motile. As the parasite grows in size the corpuscle becomes smaller and stunted with an irregular crenated margin. The protoplasm of the organism is more refractile than that of the tertian organism and hence the outlines of the pseudopodia are more easily seen, although the parasite is less actively motile. In 24 hours the red cell is quite small, crenated, and distinctly brassy in color. The organism is round or oval, quite distinct, slightly ameboid, and its pigment blackish-brown in color and gathered at the periphery, especially on one side, thus differing from the tertian organism in which the pigment is scattered throughout the organism. The pigment granules have practically no motion at this stage of the development. As development proceeds the parasite fills from one-third to one-half of the cell, becomes rounder, and loses its ameboid power. The protoplasm is very distinct and highly refractile. During the third day only a rim of the cell is left and this usually takes on a dark, brassy tone. The organism is at this time full grown and is about seven microns in diameter. The pigment now passes from the periphery of the organism toward the center in definite radial lines, giving a wheel-like formation, with the pigment granules forming the spokes. Later the pigment collects in the center and we have the formation of the presegmenter form. Following this the organism becomes opaque, refractive dots appear in a single regular circle about the periphery, and crenations of the border appear with these dots as a center. Lines of division start from these crenations and run to the center, forming from six to twelve segments, like the petals of a flower, giving rise to the name "daisy," "marguerite," or "rosette" form. These quartan segments are much more perfect than are the tertian forms, and later separate to form the hyaline types which take up the development as outlined above. This whole cycle of the development of the quartan organism takes place in the peripheral blood as it does in the tertian organism, but the number of segmenter forms is much more numerous in the quartan type than it is in the tertian form. This is probably due to the fact that a large number of the tertian forms accumulate in the internal organs.

The gamete forms are not as frequently seen as are those of the tertian organism. They are similar in appearance, but somewhat smaller than those of the tertian organism and give rise to flagellation in the same manner. The extracellular forms are occasionally found, but not so frequently as those of the tertian form.

The distinguishing marks between these two types of organisms may be summarized as follows: The cycle of development of the tertian organism is 48 hours, while that of the quartan is 72. The quartan organism is smaller, more refractile, less ameboid, and its pigment is coarser, darker, less motile, and more peripheral in position. The corpuscle infected by the quartan organism is smaller, shrunken, crenated, and more brassy. The presegmenter and segmenter stage are much more distinctive in the quartan than in the tertian type and more of the segment forms of the former are found in the peripheral blood, although the number of segments of the quartan type are less than those of the tertian parasite (Emerson).

(c) The Estivo-autumnal Parasite (*Plasmodium precox*; *Plasmodium falciparum*).

This is the most dangerous type of malarial infection. The duration of the cycle of development varies from 24 to 72 hours. In infection with this organism the members of the same group do not always develop in the same unity, so that we may find at times an intermittent fever, but one which becomes more and more continuous. The hyaline forms are similar to those of the tertian and the quartan types, but are slightly smaller and assume the "signet-ring" form much more commonly and maintain it longer. This early form of the estivo-autumnal parasite is distinguishable from the tertian by the shrinkage of the red blood-cell and from the quartan parasite by the smaller dimensions. In some cases the rings do not show the thickening of one segment, but remain of a uniform fine caliber throughout. These rings which do not show the distinct "signet-ring" type nearly always present two nuclear bodies lying at opposite poles or close together. Occasionally these rings appear as if unfolded and stretched across the cell like a thread, the nuclei appearing at irregular intervals. These rings may at times lose their refractility and become ameboid. As the parasite develops, a slight amount of pigment appears, usually seen as one or two granules which are motionless, as a rule, and are located at the periphery of the parasite or at the inner edge of the biconcavity. The cell is very commonly much shrunken, crenated, and brassy, even in the early stages. Some cells, which do not contain parasites, show the same injurious effects of the organism. The parasite at this time occupies about one-fifth of the cell. The infected cells now usually disappear from the circulation and continue their development in the lymph-glands, especially in the spleen. In some cases, however, the parasite does continue its development in the peripheral blood, but this is rare. In such blood or in that obtained from the spleen, the pigment appears much increased and seems to be rather coarse and dark in color, thus resembling very closely the quartan organism at this stage. It seems to be a general rule that the more malig-

PLATE XXX.



THE ESTIVO-AUTUMNAL PARASITE.

1. *Normal erythrocyte.*
- 2, 3. *Young hyaline ring-forms.*
- 4, 5, 6. *Intracellular hyaline forms.* In 4 the parasite appears as an irregularly shaped disc with a thinned-out central area. In 5 and 6 its amoeboid properties are obvious.
7. *Young pigmented intracellular form.* Note the extreme delicacy and small number of the pigment granules. (Compare with 6, Plate VI, and with 3, Plate VII.)
- 8, 9. *Later developmental stages of 7.*
- 10, 11, 12. *Segmenting forms.*
- 13, 14. *Crescentic forms at early stages of their development.*
- 15, 16, 17, 18, 19. *Crescentic forms.* In 15 and 19 a distinct "bib" of the erythrocyte is visible. Vacuolation of a crescent is shown in 18, and polar arrangement of the pigment in 17.
20. *Oval form.*
- 21, 22. *Spherical forms.*
23. *Flagellate form.*
24. *Vacuolation and deformity of a spherical form.*
25. *Vacuolated leucocyte apparently enclosing a dwarfed and shrunken crescent.*
26. *Remains of a shrunken spherical form.*

(E. F. FABER, *sec.*)

(From Da Costa's "Clinical Hematology.")

nant the type of estivo-autumnal malaria, the fewer older forms are seen in the peripheral blood, although numerous young parasites are present. In some cases the hemoglobin becomes concentrated around the parasite, leaving an almost colorless ring at the periphery of the cell. The cycle of development in the internal organs seems to take place within the macrophages, which are best studied in the fresh specimen. The parasite develops to about 5 microns in size, which is about half the size of the cell, and when full grown has its pigment all in the center, never diffusely scattered as in the tertian organism or peripherally located as in the quartan type. This form is rarely seen in the peripheral circulation. The segmenters vary in size from $2\frac{1}{2}$ to 5 microns in diameter, the process of segmentation being similar to that of the other organisms giving rise to the formation of 15 or 16 very small segments.

Certain characteristic forms of this type of malaria appear in the peripheral blood from about the seventh day of infection and in the internal organs as early as the fifth day. These forms are known as the *crescents* and the *ovoids*. The crescents¹ are slightly longer than the red blood-cells and show a distinctly crescentic shape with rounded ends, although irregular forms are at times observed. They are very refractile and usually show a fringe of the degenerated red blood-cell, which is more abundant in the concavity of the crescent and forms the so-called "bib." The pigment is large in amount and is massed at the center of the crescent, occasionally in the form of a sheaf or ring. The granules are usually coarse and rod-shaped. These crescents very frequently change their shapes, becoming oval, dumbbell-shaped, or circular and then may resume their original crescentic form. In the circular types no trace of the corpuscles is seen and the protoplasm is not as distinctly refractive as is that of the crescent. In this form of malaria we also find pigmented leucocytes, both the polynuclear neutrophiles and the large mononuclears assuming this function. In these phagocytic cells one may see masses of pigment or even parasites, especially the segmenting and flagellating forms. These pigmented cells are also seen in the other form of malaria, but only just after the chill, while in the estivo-autumnal form they may occur at any time during the infection.

Examination of Stained Specimens.

The technic of making preparations of malarial blood for staining is practically the same as that outlined previously. Precaution must be taken to make thin smears so that the parasite may be brought out more clearly. The stains to be used will depend largely on the experience of the worker, but the writer would recommend the thionin and Nocht stains above the others, although the Wright and Giemsa stains will frequently give beautiful pictures. Stains which have been kept for some time are not always reliable, so that it is well to have fresh specimens of the stain on hand for use. If the blood has been kept for some time before staining a diffuse plasma staining with methylene blue will be observed.

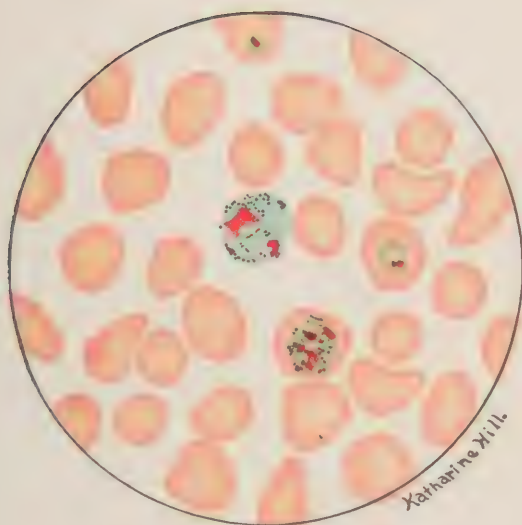
¹ See Thomson (Ann. Trop. Med. and Parasitol., 1914, VIII, 85) for a discussion of the origin and development of these crescents.

The Tertain Parasite.

The young hyaline form consists of a mass of blue protoplasm usually grouped in ring-form with a mass of reddish-violet stained chromatin, usually situated at the thinner portion of the ring and extending for a large part within the clear achromatic or vesicular part of the parasite. These hyalines are 2 to 3 microns in diameter. There is some discussion as to what portion of the parasite the name nucleus should be applied. Some give this term only to the chromatin staining part, while others include both the chromatin and achromatic portion. However this may be, it is necessary for the recognition of the tertian organism that the blue protoplasm and the red chromatin be both observed. There are frequently artefacts in the blood which resemble very closely these hyaline forms so that the worker must be constantly on his guard. Such artefacts are the Maragliano degenerations so commonly seen in red blood-cells and also the cases in which blood-platelets lie upon a red blood-cell. Any structure which lies upon a red blood-corpuscle appears surrounded by a colorless zone, while the true malarial ring is in direct contact with the hemoglobin of the cell. Moreover, such artefacts will not show the chromatin staining portion which is so characteristic of the hyaline ring. In the specimens examined at the end of 24 hours, one will observe that the achromatic area has become somewhat larger, while the chromatin portion seems to be grouped in more irregular masses, some cells appearing to have several nuclei. In the full-grown parasite the chromatin breaks up into a cluster of fine granules which are scattered diffusely through the cell in the form of strands and masses. These chromatin clumps separate into from 15 to 20 dense round masses, around which the protoplasm collects with them as a center. The protoplasm at this stage is distinctly achromatic and is always so in the segmenting cell. The distinct, achromatic, milky zone surrounds each segmentary chromatin clump, while the general protoplasm shows a diffuse faintly basic staining. The pigment which must not be confused with the chromatin is pushed toward the periphery and, after segmentation is complete, collects in masses near the center. It is to be recalled that at the time the pigment collects in the center in the fresh specimen there is no distinct evidence of segmentation, although this segmentation shows quite distinctly in the stained specimen.

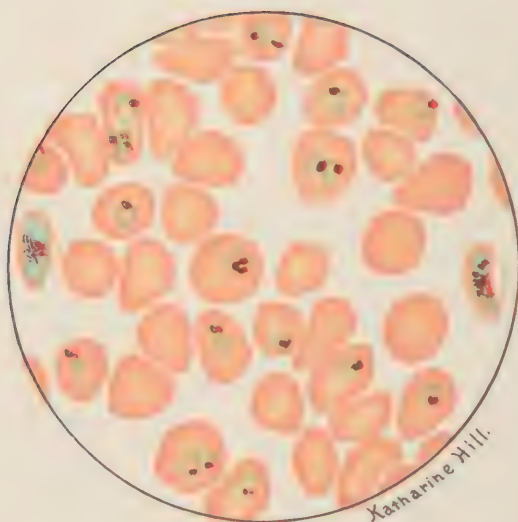
The sexual development of the parasite is easily followed in some cases in the stained specimen. According to Stephens and Christophers, the young gamete is characterized by the position of the chromatin as it lies in the center of the vacuole instead of at the edge. During the development the gamete is occasionally filled with basophile particles which are known as Plehn's karyochromatophilic granules or Schügnier's granules. The full-grown macrogamete contains an abundance of protoplasm which stains a deep blue and a small amount of chromatin in a compact mass, which is peripherally placed and surrounded by a thin vacuole-like area. The pigment of these female cells is uniformly distributed throughout the cell and the inclosing red blood-cell can be seen only with difficulty. The chromatin is much more voluminous in the microgametocytes, but is looser and centrally

PLATE XXXI.



TERTIAN MALARIAL PARASITE. (WRIGHT'S STAIN.)

PLATE XXXII.



Katharine Hill.

ESTIVO-AUTUMNAL PARASITE. (WRIGHT'S STAIN.)

placed in a large achromatic zone arranged in the form of a band which stretches clear across the cell. The protoplasm is in the form of a ring around the nucleus and stains more of a grayish-green color than does the bluish protoplasm of the macrogamete.

The Quartan Parasite.

The structure of the quartan parasite resembles very closely that of the tertian form, but in the hyaline type the chromatin mass is less distinct and is in the form of an irregular clump of granules in the older forms, while in the younger a cluster of fine granules without any distinct achromatic zone is seen. As development proceeds the parasite generally takes a form extending across the cell and usually occupies the larger portion of the red cell which has become shrunken and irregular in shape. The segment forms are much more distinct in the quartan type than in the tertian and show much more regular and geometric lines of cleavage with the chromatin exactly in the center of the crenated surface. The pigment granules are coarser and much more distinct than in the tertian form and are more peripherally located.

The Estivo-autumnal Parasite.

The hyaline forms in this type show the chromatin in two or more masses or filaments. The protoplasm is scantier than in the other forms and remains so throughout the cycle of development of this parasite. A very characteristic appearance of the hyaline rings of this type seems to be the thickening of the protoplasmic layer opposite the chromatin mass. The gamete forms are distinctly spherical, being of the same thickness all the way round. Their nucleus forms a portion of the ring, but does not project as in the schizonts (the asexual parasites). The red blood-cells in these sexual types usually show no coarse granular stippling. The crescent forms, which are characteristic of this type of malaria, show the chromatin in a loose network which occupies the larger portion of the cell, has little blue staining protoplasm, and has its pigment scattered throughout its body. This is the male form and is somewhat kidney-shaped and is shorter and broader than the female type. The female crescent is longer and narrower, its chromatin more or less compact and centrally located, its pigment in a ring around the nucleus or in a clump at the center, while its protoplasm is more or less extensive and takes a distinct bluish tinge. We also find two types of the circular form. The microgametocyte is smaller than the red cell, distinctly spherical in shape, with its chromatin in the center in a large irregular mass, or in several dense masses near the periphery. These masses containing chromatin material are later extruded and form the flagella or microgametes. The macrogamete is two or three times the size of the microgametocyte, is often of a triangular shape, and has abundant blue-staining protoplasm. The chromatin is in a single mass at the periphery and is surrounded by a circle of pigment.

The examination of the stained specimens does not give as great an opportunity for study of the developmental cycle of these parasites as does the examination of a fresh specimen. The conditions found in the fresh blood resemble more nearly those found in the stomach of the mosquito than in

the circulating blood, so that many pictures seen in the fresh specimen are practically never found in the stained slide.¹ It is further to be said that we do not always find malarial organisms either in the fresh or stained specimen, although the patient may be at the time suffering from malaria. It may be stated, as a rule, that in all well-marked initial attacks of malarial fever the parasite may be found in the blood if it be examined within 18 hours of the chill. The energetic use of quinin has so much influence upon the ameboid types of the parasites, that the blood may fail to show any of these organisms, although the patient may die from the effect of the infection. It is safe, however, to state that there is practically no case of malaria in which parasites may not be found in the blood, if frequent and repeated examinations are made.

Development of the Organism within the Mosquito (Sporogony).

The cycle of development of the malarial organism has been more closely followed in the mosquito in the case of the estivo-autumnal parasite. For any development to occur within the body of the mosquito it is necessary that the macrogamete become fertilized, so that the course in the mosquito is one of sexual development. The microgametocytes throw out their flagella (microgametes) and the macrogamete ripens in the stomach of the mosquito by casting off karyosomes (polar bodies consisting of chromatin), and in so doing causes the formation of a slight mound at one portion of the organism through which the free flagellum enters. This process occurs in from one to one and a half hours after the mosquito has bitten a patient infected with malaria. The nuclear material of the macrogamete and microgamete then unite. The cell then forms a distinct motile spindle shape called the *vermiculus* or *oökinet*. The size of this fertilized macrogamete is from 20 microns up and may be found in about 48 hours after the blood has been ingested. This motile form is found only in the stomach of the mosquito. The *vermiculus* then bores its way through the epithelial cells of the intestinal wall and becomes encysted between the intestinal epithelium and the elastic layer, which forms the membrane of the spore cyst (*zygote*, *oöcyst*, *sporoblast*). This zygote increases rapidly in size and the nucleus divides rapidly. In its growth it bulges outward from the intestinal wall forming pendulous tumors into the body cavity which growth may vary from four and a half to ninety microns in diameter. This stage is associated with the appearance of much pigment and is called the *medium zygote* or medium sporoblast stage. The protoplasm gathers around the divided nuclei, forming daughter cysts which are connected by bridges of protoplasm forming the stage known as *large zygote*, *large sporoblast*, or *large oöcyst*. In each of these divisions the nucleus divides many times, the daughter nuclei remaining on the surface of each daughter cyst. The protoplasm now collects around each daughter nucleus, the first forming spherical cells which then elongate into threads lying parallel over the remains of the sporoblasts. These threads are called *sporozoites* and have an elongated nucleus. The final length of these sporozoites is about 14 microns and their width about 1. Their protoplasm is thick, homogeneous,

¹ See Clark (Jour. Exper. Med., 1915, XXII, 427) for a discussion of the value of the study of placental blood films in the diagnosis of malaria.

and very refractive. They are sometimes present to the number of 10,000 in some zygotes, but more frequently are not so numerous. As the oöcyst becomes larger it bursts into the body cavity, the sporozoits of each cyst ripening at about the same time. These sporozoits wander at first free, but soon collect in the salivary gland of the mosquito. They are motile and move with a bending and gliding motion. When they are inoculated into the blood of man by the bite of the female mosquito they attach themselves to the red blood-corpuscle and finally penetrate it to form the initial hyaline type of the organism. The period of incubation after the bite of the mosquito is usually between the eighth and twelfth day, when the first chill will appear, although the exact time of appearance of the initial symptoms will depend upon the number of sporozoits introduced into the circulation (Emerson). The anopheles is the only type of mosquito which is at present known to be the host of the malarial organism and to give rise to the development of the *gameto-schizonts* (the sexual cells), which the bite of the female animal introduces into the blood cells which are known in their future development as schizogones. The sexual cycle within the mosquito is known as the sporogone, which Rowley-Lawson¹ has recently shown may occur in the circulating blood of the human host.

General Changes in the Blood in Malaria.

There are few conditions which lead so rapidly to such an extreme reduction in the red cells as does acute malaria.² An acute attack may reduce the red cells to as low as 500,000 cells as reported by Kelsch. Frequently a reduction of 1,000,000 is observed during the first day, with a progressive reduction as the time goes on. In the afebrile period of the disease a continuous fall is observed, but this is much less rapid. The regeneration of the cells is very active so that an increase in the number of cells has been observed directly after an attack in some cases. In cases of chronic malaria the red cells are commonly reduced to as low as 583,000 (Kelsch), while when attacks occur only at intervals and are promptly stopped by quinin no reduction in the red cells may follow (Marchiafava). In cases of moderate severity the usual changes of secondary anemia are present in the red cells. Polychromatophilia and granular degeneration of the reds progresses steadily, while the hemoglobin content of the cells may be markedly reduced. Frequently cases are seen in which the anemia takes on the absolute pernicious type, so that the parasites seem to have been massed in the bone-marrow. As Ewing states, "there can be no doubt that the tendency of the estivo-autumnal parasite to be massed in the bone-marrow, in both ameboid and crescentic phases, and the excessive demand on red-cell production arising in the disease

¹ Jour. Exper. Med., 1911, XIII, 263; Ibid., 1913, XVII, 324. See, also, King, Jour. Exper. Med., 1917, XXV, 495.

² Rowley-Lawson (Arch. Int. Med., 1912, IX, 420) believes that this severe anemia is due to the migration of the plasmodia from corpuscle to corpuscle. See James, Jour. Infect. Dis., 1913, XII, 277; Brown, Arch. Int. Med., 1913, XII, 315; Jour. Exper. Med., 1913, XVIII, 96; Rowley-Lawson (Ibid., 1914, XIX, 450 and 453; Ibid., 1915, XXI, 584) discusses the extra-cellular relation of the parasite to the red corpuscle. See, also, Lawson, Jour. Exper. Med., 1918, XXVII, 739 and 749; Ibid., 1919, XXIX, 361; Cardier, C. R., soc. biol. Paris, 1919, LXXXII, 355; Lawson, Jour. Exp. Med., 1920, XXXI, 201; Ibid., XXXII, 139.

render pernicious malaria an extremely favorable condition for this disturbance of the structure of the marrow and the development of specific megaloblastic changes." Besides the changes which can be directly referred to anemia or toxemia, changes in the size of the cell are quite constant, the tertian parasite causing from the start swelling of the cell and progressive loss of hemoglobin, while the quartan and estivo-autumnal forms cause the red cell to shrink and take on a peculiar brassy tone.

The leucocytes do not show very characteristic changes. In the acute malarial attacks of average severity the absence of leucocytosis is of considerable corroborative value, although a slight leucocytosis amounting to about 10,000 with an increase in the percentage of polynuclear cells has been observed by Billings and others. Except during the three or four hours immediately following a chill malarial blood usually shows a diminished number of leucocytes with a distinct relative lymphocytosis, which finding is that seen in typhoid fever.¹ In the more severe estivo-autumnal attacks a definite leucocytosis has been distinctly observed, especially in the hemoglobinuric or black-water type of malarial infection. The extent of the leucocytosis varies between 10,000 and 35,000, although many attacks fail to cause any distinct increase. During the afebrile periods the eosinophile cells are usually increased and may be observed throughout the course of the attack. Neutrophile myelocytes are occasionally present and rarely eosinophile myelocytes. Pigmented leucocytes are seen in the majority of cases, especially in the severe and fatal cases, the pigmented leucocytes being more closely related to the severity of the paroxysms than to the extent of the deposits in the various viscera. These pigmented or phagocytic cells include mononuclear and polynuclear leucocytes and a few endothelial cells. The large and small mononuclears usually contain pigment or rosettes, while many of the polynuclear leucocytes also contain the parasites. These phagocytes may contain, besides parasites and malarial pigment, hematoidin, hemosiderin, red blood cells, leucocytes, and occasionally an unknown crystalline pigment.

(2) Relapsing Fever (Famine Fever).

The cause of this fever is the *spirillum* of Obermeier (*spironema recurrentis*) and is not a member of the class of bacteria but belongs to the class of spirochete. This organism is between 16 and 40 microns in length and about 1 micron in width, but is subject to considerable variation in size. It is thin, sharply curved, and appears to be structureless. It takes a deep chromatin stain and also stains with methylene blue in from two to five minutes. It is seen in the blood only during the febrile period of the disease and at that time is actively motile with a rapid wavy motion, much resembling the movements of a coiled spring in its stretching and collapsing. It moves rather slowly among the corpuscles, but does not disturb them to any extent.² Cases have been reported in which these spirochete are present in the blood

¹ See Zweig and Matko, Wien. klin. Wchnschr., 1916, XXIX, 1328.

² Noguchi (Jour. Exper. Med., 1912, XVI, 199) has succeeded in cultivating this organism and shows that this pathogenicity is not thus diminished. See Plotz, Ibid., 1917, X-XVI, 37.

24 hours before the chill, but they are usually to be found in larger numbers at the time of the rise in temperature, increasing rapidly from day to day. The fever, as a rule, continues about six days, at the end of which time these parasites leave the blood. Strangely enough these organisms have been found in varying numbers in different parts of the circulation, while there does not seem to be any strict parallelism between their number and the height of the fever. Loewenthal has applied the agglutination test to the blood of suspected cases and finds the reaction positive in 85 per cent. of the cases in the periods in which the parasites are absent. Nothing of a characteristic nature is observed in the general blood picture.

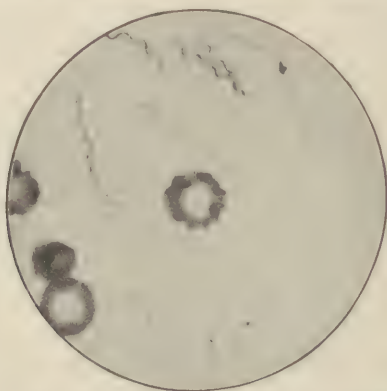


FIG. 146.—Spirillum of Obermeier. (Pilfield.)

Cases of relapsing fever are practically never found in the United States, unless imported through the medium of emigrants from Russia and especially India. The cases reported by Wellman show that relapsing fever, as found in West Africa, may arise from the bite of a tick transmitting the spirillum of Obermeier.¹

(3) Sleeping Sickness.

This very interesting condition which is so prevalent in Central and West Africa seems to be due to an actively motile fusiform flagellate known as the *trypanosoma Gambiense*, which can be found in the blood free in the plasma (never intracorpuseularly), moving with a screw-like motion among the red cells which it does not seem to disturb. This parasite doubtless has a sexual development, its host being the common fly, *Glossina palpalis*, while closely related trypanosomata are transmitted by the bites of various flies, especially one of the seven varieties of the tsetse fly. This organism is from two to three times as long as a red blood-corpuscle (18 to 25 microns) and 2 to 2½ microns wide, having a flagellum anteriorly and an undulating membrane ex-

¹ Nicolle, Blaizot and Conseil (Ann. de l'Inst. Pasteur, 1913, XXVII, 204) show that the bite of the louse does not transmit this disease as is commonly supposed. If the louse be crushed on the skin and there are abrasions on the skin from scratching, the parasites gain access to the system. The *Spirocheta recurrentis* was first cultivated by Noguchi (Jour. Exp. Med., 1912, XVI, 109) by methods similar to those for the *spirocheta pallida*. Since that time by several workers, such as Hata, Cent. Bakt., Ito, Abt., Orig., 1914, LXXII, 107; Plotz, Jour. Exp. Med., 1917, XXVI, 37; and Ungermann, Arb. a. d. k. Gesundheitsamte, 1919, LI, 114. Recently Kligler and Robertson (Jour. Exp. Med., 1922, XXXV, 303) report a very extensive study of its cultivation and of its biological characteristics.

tending its entire length.¹ In the fresh blood specimen these parasites should be looked for with only a medium magnification. These parasites vary much in number sometimes being absent for a long period and then suddenly reappearing in large numbers. Symptoms of the disease seem to bear little relation to the number of parasites in the peripheral blood, so that in some cases it may be necessary to examine the fluid in the edematous areas or even to puncture the cervical lymph-glands.² When these parasites are stained with a polychrome dye they show a rather large red nucleus about the middle, a centrosome staining intensely in a vacuole-like area near the blunt posterior end, and a line of chromatin taking a dense red stain running down



FIG. 147.—*Trypanosoma gambiense*. (Da Costa.)

the edge of the undulating membrane and terminating in the flagellum which is also stained red. The protoplasm of the body takes a distinct blue stain. The parasite contains no pigment and, therefore, obtains its nourishment from the plasma and not from the red cell.

This disease may take an acute course, but as a rule is exceedingly chronic, running for years, but becoming fatal as soon as the parasite reaches the cerebrospinal fluid. The true sleeping sickness appears only when the cerebrospinal fluid is invaded and seems to be, according to the recent work of Koch, directly amenable to treatment with atoxyl. In examining the cerebrospinal fluid for these parasites it is best gently to centrifuge the fluid for five minutes, after which the sediment may be examined under a vaselined cover-glass.

There are many other types of trypanosomata, but the Gambiense form is the more important. This is pathogenic toward man, but cannot be distinguished from the trypanosoma of the tsetse fly which is so fatal to the

¹ See Thomson and Sinton (Ann. Trop. Med. and Parasitol., 1912, VI, 331), who have succeeded in cultivating this organism. Maynard, The Trypanosomes of Sleeping Sickness, 1915; Teichmann, Deutsche Med. Wchnschr., 1916, XLII, 1437; Adams, Jour. A. M. A., 1919, LXXIII, 1696.

² Wolbach and Binger (Jour. Med. Research, 1912, XXVII, 83) show that the trypanosomes are not confined to the blood-vessels and lymphatics, but invade the tissues. See, also, Tuttle, Jour. Am. Med. Assn., 1915, LXV, 240.

horse and mule (*trypanosoma Brucei*), that of the surra disease¹ (*trypanosoma Evansi*), or that of dourine (*trypanosoma Equiperdum*).

(4) Kala-azar.

Through the researches of Donovan, Leishman, and Ross, parasites have been demonstrated in the blood which are probably directly associated with the condition known as kala-azar, tropical splenomegaly, piroplasmosis, cachexial fever, and dum-dum fever. The organism has been called the *Leishman Donovan body*, and is a small oval, round, or oat-shaped body from $2\frac{1}{2}$ to 3 microns in diameter. These bodies have a definite cell outline and contain two chromatin masses, a larger one, a nucleus which is almost round or oval and stains faintly, and a smaller bacillus-shaped centrosome which stains deeply and is directed almost at right angles to the axis of the nucleus. These two chromatin masses are both in the long axis of the cell, the larger one being at the periphery. Many of these forms are vacuolated and the outline of the cell cannot always be seen, although these two masses thus arranged are distinctive. They are easily stained with the various polychrome dyes and are best studied with the highest lenses. These bodies probably represent a stage in the development of a trypanosome as shown by the work of Leishmann and Statham. They are not found in the circulating blood as a rule, but they have occasionally been reported in the form of intracellular bodies in fatal cases. They are easily seen in the blood obtained by splenic puncture and also in the granulation tissue taken from the ulcers.² Many are found in the mesenteric lymph-glands, bone-marrow, and liver. Some of these bodies lie free, but most of them are intracellular, either in the leucocytes, endothelial, or splenic cells, and frequently in large masses in the macrophages.³

The changes in the blood are those of a moderate anemia, associated with a leucopenia with a relative and absolute increase in the number of the large mononuclears. The average leucocyte count is about 2,000.

(5) Filariasis.

This is a condition associated with the presence of filariæ in the blood (*filaria sanguinis hominis*). While many of these filariæ⁴ are known; the most common one is the *filaria Bancrofti* (*filaria nocturna*). These are from 270 to 340 microns (0.2 to 0.3 mm.) long and from 7 to 11 microns broad.

¹ Mitzmain (Philippine Jour. Sc., B, 1913, VIII, 223) shows that this disease is transmitted by the tabanid fly (*tabanus Striatus Fabricius*). A die (Indian Jour. Med. Res., 1921, IX, 255) reports the development of this organism in spleen juice and in alimentary tract of the *Cimex lectularius* (bed-bug).

² Bates (Jour. Am. Med. Assn., 1913, LX, 898) reports a case of Leishmaniosis of the nasal mucosa, the organisms being obtained from direct smears made from the ulcers. See, also, Patton Indian Jour. Med. Research, 1914, II, 492; Mackie, *Ibid.*, 510.

³ Darling (Jour. Exper. Med., 1909, XI, 515) has reported the finding of an intracellular parasite, the histoplasma capsulatum, as the cause of Histoplasmosis, an infectious disease of the Canal Zone much resembling kala-azar. The parasite is small, round or oval, 1 to 4 μ in diameter, possesses a polymorphous chromatin nucleus, basophilic cytoplasm and achromatic spaces all enclosed within an achromatic refractile capsule.

⁴ Wellman and Johns (Jour. Am. Med. Assn., 1912, LIX, 1531) have succeeded in cultivating the filaria immitis on artificial media. See Fülleborn, in Kolle and Wassermann's Handbuch der pathogen. Mikroorg., 1913, VIII, 185; Johns and Quereas, Am. Jour. Trop. Dis. and Prev. Med., 1914, I, 620; Smith and Rivas, *Ibid.*, 1914, II, 357; Dutcher and Whitmarsh, *Ibid.*, 1915, III, 65; Johnson, Southern Med. Jour., 1915, VIII, 630; Rosenberger, N. Y. Med. Jour., 1915, CII, 883; Lyon, Jour. A. M. A., 1917, LXVIII, 118; Yamada and Yamamoto, Mitt. a. d. med. Fak. Tokyo, 1917, XVII, 87; Lynch, Jour. A. M. A., 1919, LXXVIII, 760.

They are enclosed in a sheath which is considerably longer than is the parasite and shows fine cross striations. The anterior end of the parasite is abruptly rounded and has a six-tipped prepuce and a sharp fang, while the posterior end tapers for about two-fifths of the length of the parasite. The median axis of the parasite is granular. The movement of these parasites is distinctly progressive at first as seen under the microscope, but they soon become motionless, appearing to attach themselves to the glass slide at their anterior end. Strangely enough these embryos appear in the circulation only toward evening, their numbers gradually rising to a maximum about midnight and diminishing toward dawn. During the day they are found in the internal



FIG. 148.—*Filaria Bancrofti*. (Da Costa.)

organs, especially the lungs. The forms appearing in the blood are practically all embryos, as the adult types lie in the lymphatics where they obstruct the lymph flow.

The obstruction in the lymph-glands may also be brought about by the eggs, which are 25 to 38 microns long by 15 broad. The embryos reach the general circulation only through the thoracic duct. The female filaria is 85 to 150 mm. long with a distinct neck, a head with a simple, minute, terminal mouth, and a plain cylindrical body covered by a striated cuticle and tapering toward the neck and tail. The tail ends bluntly and has a small depression surrounded by two lips. The male is about 80 mm. long, without a neck and having a tendril-like tail rolled into one or more spirals.

Like the malarial organisms, the filaria has an intermediate host in the mosquito, both of the *culex* and *anopheles* variety. The embryos, which are taken up by the bite of the mosquito, cast off their sheath in about one hour in the stomach of a mosquito. Some of these embryos die at this stage, but others bore actively through the intestinal wall to the muscle, where they remain. In the next two or three days the embryo becomes larger and its alimentary tract develops. On the seventh day the worm is about $1\frac{1}{2}$

mm. long and is perfectly developed. It now travels toward the head and takes its position in the labium, whence it enters the blood of its new host during the biting by the insect. A large number of these adult forms is necessary to cause very severe cases and many years pass before any symptoms are manifest.

In examining the blood for the filaria, it is best to take a specimen late at night and to make a very thick, fresh specimen which should be examined with a low power.¹ Besides the ordinary anemia which may develop in such cases, we find a very striking eosinophilia which may run from 4 to 17 per cent.

A very characteristic finding in such cases is the condition of hematochyluria followed by chyluria. This hematochyluria seems to be due to rupture of the varicose lymph-vessels of the bladder, as these form a large part of the collateral circulation when the thoracic duct is occluded (Emerson). Such attacks may occur for years and be separated by long intervals. Their onset is spontaneous or following exertion and is usually associated with pain and fever. The urine shows the presence of blood, chyle (as high as 3.8 per cent. fat), and embryos.

Many other forms of filariæ are known, but this Bancroft type seems to be the more important. While this disease occurs endemically in the tropics there are undoubtedly many cases in this country. It is, therefore, wise in a case showing lymph tumor, elephantiasis, and hematochyluria, especially when pain and fever and enlarged spleen are present, to examine the blood for the filaria Bancrofti.

(6) Syphilis.

The search for the etiologic factor of syphilis has extended over a period of many years and various organisms have been described, from time to time. Through the work of Schaudinn and Hoffmann in 1905, a parasite was discovered which was so constantly associated with syphilitic lesions, whether primary, secondary or tertiary, that it was almost universally regarded as the causative agent in this disease. The cultivation of this organism in pure culture by Noguchi (see p. 679) and the demonstration of the pathogenic properties of this cultivated parasite have definitely established the etiologic relationship of this organism to syphilis. This organism is now called the spirocheta pallidum,² being formerly designated spirocheta pallida or treponema pallidum.

While it is now a relatively simple procedure and a matter of almost daily laboratory routine to demonstrate the presence of these organisms in the primary and secondary syphilitic lesions or in the blood of affected patients, the detection of the spirochete in the tertiary stages or in the parasyphilitic conditions is not by any means always successful. It is in the latter types of this disease that the Wassermann and luetin tests are so frequently called upon to settle the diagnosis, as the primary and secondary stages are more amenable to direct diagnosis.

¹ The concentration method of Smith and Rivas is of advantage. This consists of adding 1 c.c. of blood to 10 c.c. of 2 per cent. acetic acid, centrifuging, washing and centrifuging several times, spreading the sediment on a slide and examining with low power lens.

² According to the designation adopted by the Medical Research Council.

It has, of course, been known for some time that chronic nervous conditions, such as general paralysis and tabes dorsalis, were closely associated with long-standing syphilitic infection, but this relationship remained to be clearly proven. Noguchi and Moore¹ have demonstrated the presence of the spirochete in the brain tissue of paretics and also in the posterior columns of the spinal cord in tabes. These results have been abundantly confirmed by others. It is true that the results show only about 25 per cent. of positive findings in these cases but this is not surprising in view of the latent character of the infection. Such a result indicates, merely, the association of these organisms with the pathologic lesions of the disease and does not prove their absolute etiologic relationship to syphilis in general. Later work by Noguchi and others has clearly shown that typical syphilitic lesions may be caused in rabbits by inoculation of an emulsion of the brain of a parietic individual. The results show about the same percentage of successful inoculations as do those obtained from direct histologic examination of the tissues.² Graves³ goes even a step further and believes that his experiments show that rabbits may be infected with syphilis directly from the blood of general paretics. The proof is, therefore, absolute that these diseases, general paralysis and tabes dorsalis, are the result of actual syphilitic infection of the brain and spinal cord. It is evident, therefore, that the Wassermann and luetin tests can be of inestimable service in such cases in the way of clearing up a diagnosis.

The spirochete *pallidum*⁴ derives its name from its low refractive power and the difficulty with which it takes up anilin dyes. It has a very delicate structure, usually presenting 10 to 40 deep spiral incurvations in the larger specimens or only a few in the smaller ones. Its length varies between 4 to 10 microns and its width does not exceed $\frac{1}{2}$ micron. Noguchi has divided the strains of this organism into a thick, a thin and a medium type. The organism has been demonstrated in the circulating blood, in the scrapings obtained from the chancre, in the incised papules, in smears from the mucous patches, and in the fluid aspirated from the inguinal glands. It seems to be easily demonstrable in the blood from a splenic puncture, while in the congenital forms it is found in the internal organs and in the peripheral blood. A characteristic difference between this and some other organism types (*spirocheta buccalis*), with which it might be confused, is that its ends lie above and below a longitudinal line drawn through the center of its curvatures, while in the other forms the ends lie on the projection of such a

¹ Jour. Exper. Med., 1913, XVII, 232; Noguchi, Jour. Am. Med. Assn., 1913, LXI, 85; Münch. med. Wchnschr., 1913, LX, 737; Jour. Cutan. Dis., 1913, XXXI, 543; Forster and Tomaszewski, Deutsch. med. Wchnschr., 1914, XL, 694; Warthin, Am. Jour. Med. Sc., 1916, CLII, 508; Keily, Jour. Lab. and Clin. Med., 1917, II, 260.

² See Hoche, Med. Klin., 1913, IX, 1065; Forster and Tomaszewski, Deutsch. med. Wchnschr., 1913, XXXIX, 1237; Wassermann, Ibid., 1281; Levaditi, Marie and Bankowski, Ann. de l'Inst. Pasteur, 1913, XXVII, 577; Nichols and Hough, Jour. Am. Med. Assn., 1913, LXI, 120; Wile, Ibid., 866; Wile and De Kruif, Jour. A. M. A., 1916, LXVI, 646; Reasoner, Ibid., 1916, LXVII, 1799; Wile, Jour. Exper. Med., 1916, XXIII, 199; Grannelli, Policlinico, 1917, XXIV, 3.

³ Jour. Am. Med. Assn., 1913, LXI, 1504.

⁴ See McDonagh, Brit. Jour. Dermatol., 1912, XXIV, 381; and Ross, Lancet, 1912, II, 1105. See Noguchi, Spirochetes (Harvey Lecture), Jour. Lab. and Clin. Med., 1917, II, 365 and 472; Am. Jour. Syph., 1917, I, 261.

line.¹ The organism moves in an oscillatory manner about its longitudinal axis, its movements being winding, bending and whipping, while in the spirilla the longitudinal axis remains rigid.² Schaudinn demonstrated the existence of a flagellum at each end, while the other spirochetæ have an undulating membrane.

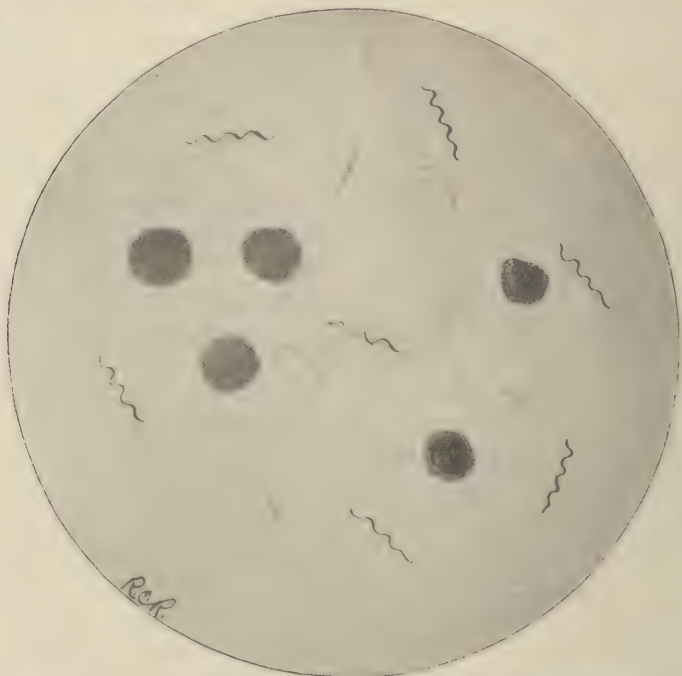


FIG. 149.—*Spirocheta pallida* and refringens. (Pitfield.)
The darker ones are the refringens.

These organisms are seen only with great difficulty in the specimens of fresh blood, but thanks to the introduction of the ultra-condenser (the dark-field illuminator) we are in a position to see these organisms, both in the splenic and peripheral blood, although considerable practice is necessary properly to adjust the light.³ These organisms do not take anilin dyes readily, so special methods have been advanced for their demonstration in smears.

¹ Ciarla (Policlinico., 1917, XXIV, 369) calls attention to the fact that the spirals are extremely regular and appear to be coiled around a central axial filament whose demonstration is doubtful.

² Zinsser and Hopkins (Jour. A. M. A., 1914, LXII, 1892) have shown that the spirocheta may live and remain motile 11½ hours on wet towels exposed to room temperature. Reasoner (Ibid., 1917, LXVIII, 973) shows that soap, as applied in shaving, destroys the motility of these organisms at once. The importance of these observations as to possibility of transmission of syphilis by barbers is evident.

³ See Sidwell and Smith, Ill. Med. Jour., 1914, XXVI, 418. See, Special Report Series No. 19, of Med. Res. Com. National Health Insurance, London, 1918, for a full discussion of the principles of dark field illumination. Eberson (Arch. Dermat. & Syph., 1920, I, 638; Ibid., 1921, III, 111) calls attention to the fact that it is possible to have artefact "spirochetes" in these dark field specimens, extremely tenuous, filamentous forms resembling spirocheta pallida in motility and spiral structure. He believes that they are derived from the red corpuscles and may be produced at will, influences such as H-ion concentration of solutions, tonicity and transfer from the usual environment, etc. being sufficient for the demonstration of their appearance. Such possibilities should be borne in mind.

A very good stain for them is the Goldhorn stain. The smears are fixed with pure methyl alcohol for 15 minutes and then covered with the stain (polychrome methylene blue) for three to five seconds, when the excess is drained off. The specimens are then slowly introduced into clean water with the film sides down. Keep the slide in this position for four to five seconds and then shake in the water to remove the excess of the dye. The spironema appear of a violet color. This violet tint may be changed to a bluish-black by covering the specimen with Gram's iodine solution for 15 to 20 seconds, after which it is washed and dried as usual and the examination made with the immersion lens. The writer has also found the use of the Giemsa stain very reliable, especially when the staining is continued for 18 hours (see Exudates). Other stains, such as that of Levaditi, have been advocated, but they do not seem to give any better results and are more

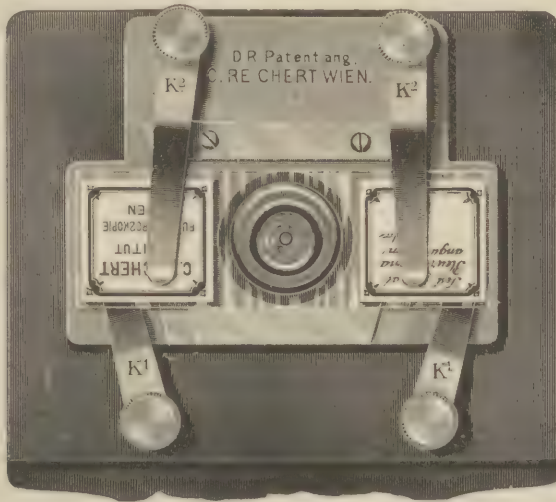


FIG. 150.—Ultra-condenser of Reichert.

complicated. For staining the spironema in tissues the Levaditi stain is admirable.

The examination of the blood is very often disappointing, owing to the fact that few spironema may be present in the specimen. Better results are obtained by examination of specimens from a curettage which has been carried sufficiently far to allow serum to appear. This serous fluid is then spread upon slides and treated in the usual manner (see Exudates).

Cultivation of *Spironema Pallidum*.

As previously stated, the causative factor of syphilis has been definitely settled only by fulfilling Koch's postulates regarding pure cultures and the production of the disease by means of these pure cultures. Schereschewsky,¹ Mühlens² and Hoffmann³ were able to cultivate the pallidum but were

¹ Deutsch. med. Wchnschr., 1909, XXXV, 835, 1260 and 1652; Ibid., 1913, XXXIX, 1408.

² Deutsch. med. Wchnschr., 1909, XXXV, 1261; Klin. Jahrb., 1910, XXIII, 339.

³ Ztschr. f. Hyg. u. Infectkr., 1911, LXVIII, 27.

unable to reproduce syphilitic lesions by means of their cultures. Bruckner and Galasesco¹ and Sowada² reported the successful reproduction of the lesions by injection of their "young impure cultures" but, as neither investigator was able to grow a second generation of these so-called cultures in any medium, their results are questionable. Recently Noguchi³ has succeeded not only in producing many generations of pure cultures of the spironema but, also, in reproducing the syphilitic lesions by use of these pure cultures. His work is of the greatest importance as it absolutely establishes the spironema pallidum as the etiologic factor of syphilis.

His technic is as follows: As the material for obtaining the cultures he uses the spironema-containing testicular tissue of rabbits, which have been inoculated with human syphilitic material. From this first generation in rabbits any number of generations may be derived by transmitting the strain, at appropriate intervals (four to six weeks), from rabbit to rabbit. By this means the spironema are almost free from banal bacteria.

The culture media is the following: 15 c.c. of a mixture of equal parts of ascitic fluid and bouillon are placed in tubes 20 cm. long and 1.5 cm. wide. These tubes are then sterilized by fractional sterilization at 100°C. for 15 minutes on each of three successive days. Then a small piece of freshly removed sterile rabbit tissue (preferably kidney or testicle, although heart muscle may be used but not liver) is placed in each tube which is then incubated at 37°C. for two days and examined for sterility. To each tube a layer of sterile paraffin oil is added to shield the medium from contact with air and to prevent evaporation. The method of cultivation must be strictly anaerobic in obtaining the first generation of spironema. Noguchi employs a combination of hydrogen gas, vacuum and pyrogallic acid in an anaerobic apparatus, which is fully described in his article. He believes the following conditions essential in obtaining his first generation: (1) the presence of suitable fresh sterile tissue in serum-water, (2) strict anaerobiosis, (3) a slightly alkaline reaction as furnished by the serum and tissue, and (4) a temperature of about 35 to 37°C.

When once adapted to the artificial ascitic-broth tissue medium, the pallidum grows well under less strictly anaerobic conditions. In fact, certain strains even grow well in a medium containing agar, provided suitable fresh tissue is placed low down in a high column of medium (serum-water or serum agar), and is covered with paraffin oil. Out of ten strains of spironema pallidum, six have been cultivated in the above medium. The difficulty in this work seems to be to obtain the first generation. The culture may be purified by permitting it to grow through a Berkefeld filter, which the pallida pass about the fifth day. Certain strains grow together with bacteria along the stab canal in a serum-agar tissue medium. But while the bacteria do not

¹ Compt. rend. Soc. de biol., 1910, LXVIII, 684.

² Deutsch. med. Wchnschr., 1911, XXXVII, 682; Med., Klin., 1914, X, 161.

³ Jour. Exper. Med., 1911, XIV, 99; Ibid., 1912, XV, 90; Ibid., 201; Ibid., 1912, XVI, 211. See, also, Nichols, Ibid., 1914, XIX, 362; Hartwell (Jour. Am. Med. Assn., 1914, LXIII, 142) reports the isolation of this organism from the blood. Zinsser, Hopkins and Gilbert, Jour. Exper. Med., 1915, XXI, 213; Akatsu, Ibid., 1917, XXV, 375; Noguchi and Akatsu, Ibid., 765; Brown and Pearce, Jour. Exp. Med., 1921, XXXIV, 185; Haythorn and Lacy, Jour. Inf. Dis., 1921, XXIX, 386.

grow out into the surrounding medium, the pallida grow out gradually in a pure state. The characteristics of this pure culture may be obtained from the literature.¹

The morphology and motility are quite typical, difficulty being experienced in distinguishing these points from those of specimens taken from human lesions. These pure cultures produce typical lesions when injected into animals, thus completing the chain of evidence.²

(7) Yellow Fever.

This infectious noncontagious disease is caused by a specific organism discovered by Noguchi and named the *Leptospira icteroides*. That the disease was blood-borne was known from the fact that injection of blood from a yellow-fever patient into healthy subjects caused the disease. Further, the organism was known to be capable of passage through a Berkfeld filter. The various organisms, which have, in times past, been held responsible for this disease, among them the Bacillus X of Sternberg, the Bacillus icteroides of Sanarelli and the myxococcidium stegomyiæ of the Yellow Fever Commission, must now be abandoned in view of the work of Noguchi and his associates.

In 1881 Finlay advanced the hypothesis that yellow fever was transmitted to man only through the bite of a mosquito of the Culex group, the stegomyia fasciata. The United States Commission, consisting of Reed, Carroll, Agramonte, and Lazear, furnished the experimental proof that this hypothesis was valid and showed that the unknown organism of the disease required a period of 12 days' development in the body of the mosquito before it could be transmitted from the stegomyia as an infecting agent. A second U. S. Commission, consisting of Parker, Pothier, and Beyer with the help of Smith stated in 1903 that yellow fever was due to a parasite of the sporozoan type, the myxococcidium stegomyiæ, which developed in the stegomyia. This organism has never been found in the human body, hence its schizogony (asexual development) is unknown. The French Commission, Marchoux, Salimbeni, and Simond, as well as Schaudinn and Carroll, do not believe that this organism has anything to do with yellow fever.³ Seidelin⁴ has reported the finding

¹ See Meirowsky ("Studien über die Fortpflanzung von Bakterien, Spirillen und Spirochäten," Berlin, 1914) for a discussion of the proper classification of this organism.

² In his later work Noguchi has cultivated the organism directly from the lesions in man, using a high cylindrical layer of solid media consisting of two parts of 2 per cent. slightly alkaline agar and one part of ascitic or hydrocele fluid, at the bottom of which has been placed a fragment of sterile tissue. The small pieces of tissue taken from the lesion are inserted deep into this media and the growth carried on as outlined above. See, also, Baeslack, Jour. Infect. Dis., 1913, XII, 55. Noguchi has, also, cultivated various types of spirochetæ, which closely resemble the pallida, and has thus shown their morphological variations (Jour. Exper. Med., 1912, XV, 466; 1912, XVI, 194, 199, 261 and 620; Ibid., 1913, XVII, 89). See also, Clark and Gates, Am. Jour. Dis. Child., 1915, IX, 126; Zinsser Hopkins and Gilbert, Jour. Exper. Med., 1915, XXI, 213. Zinsser and Hopkins (Ibid., 576) as, also, Kismeyer (Deutsch. med. Wchnschr., 1915, XLI, 306) have shown the presence of anti-bodies (agglutinins) for the treponema pallidum in the blood of syphilitics. See Zinsser and Hopkins, Jour. Exper. Med., 1916, XXIII, 323; Zinsser, Hopkins and McBurney, Ibid., 329 and 341; Ibid., XXIV, 561; Kolmer, Broadwell and Matsunami, Ibid., 333.

³ See Yellow Fever, Senate Document No. 822, Washington, 1911.

⁴ Jour. Path. and Bacteriol., 1911, XV, 282; Otto, in Kolle and Wassermann's Handbuch der pathogen; Mikroorg., 1913, VIII, 623. See, also, Wenyon and Low, Jour. Trop. Med. and Hyg., 1914, XVII, 369; Ibid., 1915, XVIII, 55; Camerer, U. S. Naval Med. Bull., 1915, IX, 65.

of a parasite, a protozoon, in the blood, which he believes the causative factor.

In 1918 the Yellow Fever Commission of the International Health Board, consisting of Drs. A. I. Kendall and Charles A. Elliott and Mr. H. E. Redenbaugh of Northwestern University Medical School, Chicago, Ill.; Dr. Mario Lebrede of Las Animas Hospital, Havana, Cuba; and Dr. Hideyo Noguchi of the Rockefeller Institute for Medical Research, New York, undertook the study of this disease in Guayaquil. The work of this Commission and especially of Noguchi¹ has demonstrated that the etiologic factor in this disease is the *Leptospira icteroides*, an organism differing from both the spirochete and treponeme but closely related to the organism of Weil's disease (the *Leptospira icterohemorrhagica*).² This organism has been recovered from the blood of infected patients, has been transmitted direct from patients to animals (guinea pigs), has been found in the blood of infected animals and obtained in pure culture. The further link in the chain has been recently published by Noguchi in the transmission of the infective agent from an infected patient, through the bite of the *stegomyia calopus*, to animals, which animals manifested the symptoms of the disease and from which the organism was obtained and cultured.

This organism, which occurs in the blood and tissues in yellow fever patients, as well as in those animals experimentally infected with the blood or tissue of yellow fever patients, is an extremely delicate filament, measuring about 4 to 9 microns in length and 0.2 of a micron in width along the middle portion. It tapers gradually toward the extremities, which end in immeasurably sharp points. The entire filament is not smooth but is minutely wound at short and regular intervals, the length of each section measuring about 0.25 of a micron. The windings are so placed as to form a zigzag line by the alternate change of direction of each consecutive portion at an angle of 90 degrees. It is unrecognizable by translucent light but becomes quite visible under dark-field illumination. It possesses an active motility, consisting in vibration, rotation, rapid bipolar progression, and sometimes twisting of parts of the filament. When it encounters a semisolid substance it penetrates the latter by a boring motion, and while passing through it the body assumes a serpentine aspect. It manifests remarkable flexibility to almost any angle while changing its course of progression in a semisolid medium. In a fluid medium, one end is usually bent in the form of a graceful

¹ Jour. Exper. Med., 1919, XXIX, 549, 565, and 585; Ibid., Ibid., XXX, 1, 9, 13, 87, 95, and 401; Jour. A. M. A., 1919, LXXII, 187; Lebrede, Vida Nueva, 1919, XI, 145; Gorgas, Carter and Lyster, Southern, Med. Jour., 1920, XIII, 873; Connor, Jour. Am. Med. Assoc., 1920, LXXIV, 650; Ibid., LXXV, 1184; Noguchi, Jour. Exp. Med., 1920, XXXI, 135 and 159; Ibid., 1920, XXXII, 381; Noguchi and Kligler, Ibid., 601; Ibid., 1921, XXXIII, 239 and 253; Grovas, Jour. Am. Med. Assoc., 1921, LXXVI, 362; Noguchi, Am. Jour. Hyg., 1921, I, 118; Jour. Am. Med. Assoc., 1921, LXXVII, 181; Guiteras, Ibid., 397.

² The close resemblance of these organisms, the fact that the anatomic lesions found in guinea pigs infected with the *Leptospira icteroides* are almost identical with those found in Weil's disease, and the fact that both yellow fever and infectious jaundice are found in the same localities, have lead some to doubt that the etiologic factor in yellow fever has really been discovered. In this connection it should be pointed out that the epidemiological and clinical features of these two diseases are quite different and that the method of transmission of each varies, yellow fever by the mosquito and infectious jaundice through the rat.

hook, and, while rapidly rotating, the organism proceeds in the direction of the straight end, the hooked end apparently serving as a sort of propeller. Many specimens are seen with both ends hooked, the organism then rotating in a stationary position unless one hook is larger and more powerful. The rapid rotation makes the organism appear like a chain of minute dots.

This organism is difficult to stain with ordinary aniline dyes, but can be made distinct by osmic acid fixation and staining with Giemsa or Wright stain. When stained with Fontana's method (see Exudates) the organism appears as a moderately heavy, slightly undulated filament without a clear elementary indentation. The peculiar forms resembling the letters C and S are quite characteristic. Specimens fixed with methyl alcohol seldom retain the elementary spirals. From these findings it is evident that the present organism falls in the general order of so called spirochetes, but in the strict sense of the term it is neither a bacterium, a spirochete, a spiro-nema, nor a treponema, but belongs to the genus *Leptospira*. For methods of cultivation see the original literature.

As this disease is beyond question blood-borne, its hematological changes are of some interest. Jones shows that anemia is infrequent, that fibrin formation is deficient, that the globucidal action of the serum is greatly increased, and that both cholemia and hemaglobinemia occur. The red cells show little variation in number, Pothier never finding them below 4,280,000. The hemoglobin suffers considerable loss, being usually between 50 and 75 per cent. This loss is restored very slowly in convalescence. Albertoni draws attention to the lack of parallelism between the percentage of hemoglobin and the specific gravity of the blood, the latter falling much more than the former. Morphological changes in the reds are rare. An occasional normoblast may be seen.

The leucocytes range between 4,660 and 20,000, the higher the count the more favorable the prognosis. In this leucocytosis the polynuclear neutrophiles are in higher proportion, only rarely being normal.¹ Eosinophiles are few and myelocytes only occasional.

(8) Infectious Jaundice.—Weil's Disease.

Although infectious jaundice had been known for some time to exist among troops, sewer workers, miners, and rice planters (especially in Japan), it remained for Weil in 1886 to describe this entity in such a way that the disease is commonly known, today, as Weil's disease. It is characterized by sudden onset with malaise, chills, high fever, intense muscular pains, jaundice and evidences of acute nephritis, such as the appearance of albumin and casts in the urine; severe cases may be accompanied by epistaxis, skin and submucous hemorrhages, and lymphadenitis.

This disease prevails rather extensively in Japan, so that opportunity for its study there was excellent. In 1914, Inada, Ido, Hoki, Kaneko, and Ito transmitted this disease to guinea pigs by inoculating them with the blood

¹ Noguchi states that the number of leucocytes soon returns to normal and in a few days a marked leucopenia sets in.

of patients suffering from the disease.¹ They were, further, able to discover in the blood and various organs of the experimental animals, a new spirochete, to which they gave the name *Spirocheta icterohæmorrhagica* and which may be established as the causative agent of this disease. Some time later Uhlenhuth and Fromme² and, independently, Hübener and Reiter³ accomplished the same thing, the latter workers naming their organism, *Spirocheta nodosa*. Still later Stokes and Ryle⁴ succeeded in transmitting the disease to guinea pigs by inoculating them with blood from the infected British soldiers on the Flanders' front, but Stokes, Ryle, and Tytler⁵ did not decide the question of the identity of their organism with that of Inada and his associates. Many workers on the French front demonstrated the presence of these spirochete in their cases and regarded them as closely related to the Japanese type.⁶ On the Italian front Monti⁷ has arrived at the same conclusion. According to Noguchi, "In America, especially the United States, there have been few epidemic or endemic cases of infectious jaundice reported from various quarters of the continent (Toronto, Middle Western and Southern United States) and from Cuba, but it was not known whether or not these cases corresponded with those found in Europe and Asia." As this disease appears to be widespread, especially in times of war, it is important that its etiology be understood and capable of recognition. The work of Noguchi has shown that these various strains are probably identical.

Although Inada and his coworkers first isolated, cultivated, described and named the etiologic factor of Weil's disease, yet the description of Noguchi, based on his studies, is so much fuller, that the following is quoted from his discussion of the subject.⁸ The elementary structure of this

¹ Jour. Exper. Med., 1916, XXIII, 377. See, also, Ito and Matsuzaki, Ibid., 557, Ido, Hoki, Ito, and Wani, Ibid., 1916, XXIV, 471; Inada, Ryokichi, Ido, Yutaki, Hoki; Rokuro, Ito, Hiroshi, and Wani, Ibid., 485.

² Med. Klin., 1915, XI, 1202, 1204, 1296, and 1375; Ztschr. f. Immunität., 1916, XXV, 317.

³ Deutsch. Med. Wchnschr., 1915, XLI, 1275; Ibid., 1916, XLII, 1; See, also, Reiter, Ibid., 1283.

⁴ Jour. Royal Army Med. Corps, 1916, XXVI, 286.

⁵ Lancet, 1917, I, 142.

⁶ Martin and Pettit, Presse Méd., 1916, XXIV, 569; Bull. de l'Acad. Med., 1916, LXXVI, 247; Costa and Troisier, C. R. soc. biol. Paris, 1916, LXXIX, 1038; Bull. et mem. Hop. Paris, 1916, XL, 1928; Merklen and Lioust, Ibid., 1865; Garnier and Reilly, Ibid., 2240; Ameuille, Ibid., 2281; Renaux, C. R. soc. biol. Paris, 1916, LXXIX, 947; Salomon and Neveu, Ibid., 1917, LXXX, 272.

⁷ Boll. Soc. med. chir. Pavia, 1916; Policlinico, 1917, XXIV, 290; Moreschi, Ibid., 265; Micheli and Satta, Ibid., 268; Trincas, Ibid., 271; Pontano, Ibid., 277; Merighi, Ibid., 280. For other cases, showing the wide-spread character of this disease, see Reiter, Ztschr. f. klin. Med., 1910, LXXXVIII, 459; Pagniez, Bull. Méd., 1910, XXXIII, 720; Lortat-Jacob and Deglaire, Bull. de la Soc. Méd. des Hôp., 1910, XLIII, 1077; Granier and Reilly, Ibid., 1128; Villaret, Benard and Dumont, Ibid., 1920, XLIV, 903; Meneirer and Durand, Ibid., 1063; Pagniez, Bull. Méd., 1920, XXXIV, 776; Sobernheim, Deutsch. med. Wchnschr., 1920, XLIV, 1160; Garnier, Médecine, 1920, II, 188; Ryle, Quart. Jour. Med., 1921, XIV, 139; Arce, Cronica Médica, 1921, XXXVIII, 65; Dargein and Plazy, Bull. de la Soc. Méd. des Hôp., 1921, XLV, 326; Manteufel, Deutsch. med. Wchnschr., 1921, XLVII, 461; Basile, Policlinico, 1921, XXVIII, Med. Soc., 211; Griffith, Jour. State Med., 1922, XXX, 70; Villaret, Benard and Blum, Bull. de la Soc. Méd. des Hôp., 1922, XLVI, 225; Wadsworth, Langworthy, Stewart, Moore, and Coleman, Jour. Am. Med. Assoc., 1922, LXXVIII, 1120.

⁸ Jour. Exper. Med., 1917, XXV, 755; Ibid., 1918, XXVII, 575, 593, and 609. See Otteraaen, Jour. Infect. Dis., 1919, XXIV, 485.

organism is that of a closely wound slender cylindrical thread with gradually tapering ends, averaging 9 by 0.25 micron. Individuals of 3 to 4 μ or 20, 30 and even 40 μ are met with in culture. The number of coils is greater in a given length than of any spirochete studied. It is so closely wound that within 5 μ there are 10 to 12 coils. Near the extremities the coils become closer. They are never very deep, the aspect of the whole body being that of a transversely barred chain of streptococci. The winding is rarely seen distinctly, although it can be brought out well by a carefully fixed stained preparation (see Exudates) or under powerful dark-field illumination. The movement and customary position of the organism in free space are characteristic. Active specimens show a straight body with one or both ends curved in the form of a semi-circle. The length of the hook at the end varies somewhat, but is usually about 3 to 5 μ . While in motion, the organism, without relaxing its elementary minute windings, rotates around its axis, making about two to four turns per second, giving the impression of a drawn out figure eight. The movement is bipolar, and its direction alternates at short intervals. When passing through a semisolid medium, the body of the spirochete assumes a wavy spiral, the movements being brusque and erratic. There is a distinct halo around the organism, but no membrane has been demonstrated. No minute flagellum-like projection could be demonstrated by staining.

The hooked ends form one of the most characteristic poses of the organism while rotating on its axis in a free space, but as soon as it meets a solid or semi-solid obstacle, it begins to penetrate into it. It is devoid of a terminal filament such as is characteristic of a spirochete, and is resistant to 10 per cent. saponin solution, unlike all other known spirochete. For these reasons, Noguchi believes it should be called *Leptospira icterohæmorrhagica*.

The finding by Noguchi of this organism in American wild rats and his identification of it with the strains observed in Asia and Europe seem

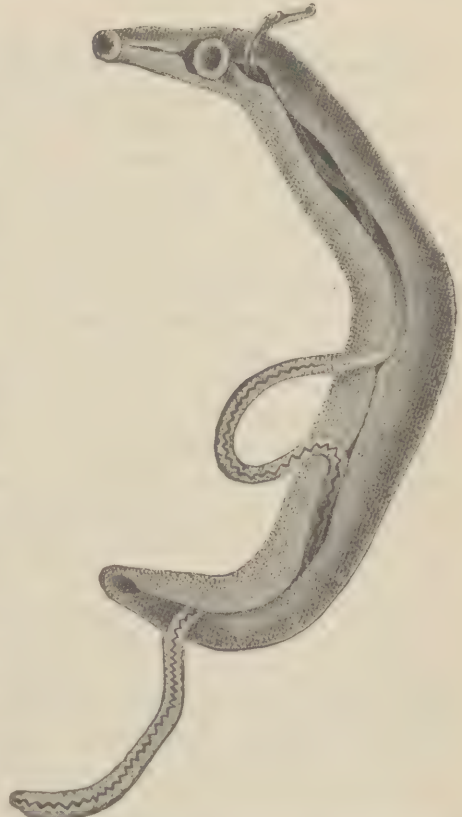


FIG. 151.—*Schistosomum hematobium*; male with female in gynecophoric groove. (Tyson after Loos.)

to indicate the source of infection in these cases. As the rats examined carried this organism in their kidneys and as the urine of infected animals, as well as of men, contains large numbers of these spirochete, it is reasonable to infer that infection may arise from the contaminated soil, the organism gaining entrance into the body through the feet. This is especially prone to occur with the bare-footed workers in rice fields in Japan and with soldiers, whose shoes have been worn through by the exigencies of the campaign.¹

(9) Rocky Mountain Spotted Fever (Tick Fever).

This disease is not to be confused with typhus fever or epidemic cerebrospinal meningitis, to both of which the term "spotted fever" has been occasionally applied.

Wilson and Chowning have reported that the blood of man affected with Rocky Mountain spotted fever shows the presence of an erythrocytic parasite which they call *Piroplasma hominis*. These parasites are ovoid in form, have ameboid motility, and are unpigmented. Three forms of these intracellular ovoids were found: (1) a small, non-motile form, 1 to 2 microns in length by 1 micron in width; (2) a larger actively ameboid form, 3 to 5 microns in length by 1 to 1.5 microns in width, and showing a dark granular spot at one end; (3) a twin form, consisting of two pear-shaped bodies, lying with their tapered ends approaching and bearing a granular spot at each end. These bodies stain best with the polychrome dyes.

Through the work of Ricketts and King it has been definitely established that the parasite of spotted fever finds its host in the wood tick (*dermacentor venustus* (Banks) or, as Stiles states, the *dermacentor andersoni*). There is apparently no cycle of development in the tick as an intermediate host. Ricketts advances much evidence against the piroplasma as the causative factor, but is unable to say that such might not be the etiologic unit. After much research he² succeeded in finding an extra- and intra-cellular pleomorphic polar staining bacillus, which is extremely small and is constantly present in the blood of infected animals as well as in the infecting tick. Whether

¹ Noguchi, Jour. Exp. Med., 1918, XXVII, 609. Foulerton (Jour. Path. & Bact., 1919, XXIII, 78) points out that, although it is not quite certain whether the spirochete of jaundice has been identified in the intestinal contents of the healthy rat, it has been found in the feces of infected guinea-pigs; and its presence in the feces of human cases may be assumed. He adds that the possible roles of insects as accessory or alternative carriers cannot be excluded. It is to be remembered that this infection through the rat is quite distinct from another type of infection through this rodent, namely, the so-called "rat-bite fever," which appears to be caused by the *spirochete morsus-muris*, as found by Futaki and his associates, although Schotmüller believes the causative organism to be a streptothrix, the *streptothrix muris rattii*. In this connection see Schotmüller, Dermat. Wehnschr., 1914, LVIII, 77; Blake, Jour. Exp. Med., 1916, XXIII, 39; Tileston, Jour. Am. Med. Assoc., 1916, LXVI, 995; Tunnicliff, Ibid., 1906; Fitaki, Takaki, Taniguchi and Osumi, Jour. Exp. Med., 1916, XXIII, 249; Ibid., 1917, XXV, 33; Ishwara, Ohtawara and Tamura, Ibid., 55; Kusama, Kobayashi and Kasai, Kitasato Arch. Exp. Med., 1919, III, 131; Arkin, Arch. Int. Med., 1920, XXV, 94; Adachi, Jour. Exp. Med., 1921, XXXIII, 647; Cazamian, Bull. de la Soc. Méd. des Hôp., 1921, XLV, 268; Randolph, Brit. Med. Jour. 1921 I, 76.

² Jour. Am. Med. Assn., 1909, LII, 379.

this is the true etiologic factor is still unsettled, but Ricketts believed it was.¹

The red cells in this disease are reduced to about 4,000,000, while the hemoglobin content may be as low as 50, thus giving a low color index. Degenerations and atypical staining qualities are rare. The leucocytes are increased to 12,000 or more and show nothing differentially abnormal, except a slight increase of the large lymphocytes.

(10) **Distomiasis (Bilharziasis).**

This is a chronic parasitic disease due to the deposition in the tissues of the eggs of the worm, *Schistosomum hematobium*, also called *Bilharzia hematobia*, *gynecophorus*, *distomum hematobium*, *distoma capense*, and *Thecosoma*. It is a very common condition in Africa, but has been found but six times in America, according to O'Neil. Infection appears to be more commonly induced by drinking unfiltered infected water, but occasionally it may come through the skin. Leiper has found that the snail is the intermediate host of this parasite.²

The adult parasites inhabit the blood of the portal vein and the vessels of the pelvis, rectum, and bladder. The male is smaller and thicker than the female, is 12 to 15 mm. long and 1 mm. broad, is flat and so folded as to form a gynecophoric canal which holds the female. The female is about 20 mm. long and 0.25 mm. thick and is the active agent in the infection.

The eggs of the parasite are oval or spindle-shaped, measure about 0.16 mm. in length and 0.05 mm. in breadth, and have a distinct spine-like projection from the posterior end or from one side. These ova are particularly frequent in the urine of such cases. Occasionally they may be found in the circulating blood.

VI. BACTERIOLOGY OF THE BLOOD

From the standpoint of differential diagnosis and treatment of a condition known or thought to be of infectious origin, it is frequently of especial importance carefully to examine the blood for the presence of various bacteria. Moreover, in many conditions, as for instance in typhoid fever, cultures made from the circulating blood often give positive findings long before other tests

¹ See McClintic (Pub. Health Rep., 1912, XXVII, 732) for an excellent bibliography of the subject; also, Rucker, *Ibid.*, 1912, XXVII, 1465; Wolbach (Jour. Med. Res., 1916, XXXIV, 121; XXXV, 147; and 1918, XXXVII, 499; 1919, XLI, 1) confirms the findings of Ricketts. See, also, Michie and Parsons, *Med. Record*, 1916, LXXXIX, 265. Wolbach (Jour. Med. Res., 1919, XLI, 1) finds that this parasite in sections invariably has the form of a minute paired organism, with the distal ends tapered so that they may be likened to a pair of diminutive pneumococci. In smear preparations of tissue one finds, in addition to the lanceolate forms, slender rod-shaped types, some of which exhibit polar granules. In stained specimens of blood the organism has the form of two somewhat lanceolate chromatin staining bodies separated by a slight amount of eosin-staining substance and surrounded with a considerable amount of pale bluish-staining material. Wolbach suggests for this organism the name *dermacentroxenus rickettsi*.

² See Looss, in Kolle and Wassermann's *Handbuch d. pathogen. Mikroorg.*, 1913, VIII, 1; Kartulis, *Ibid.*, 23; Bovaird and Cecil (*Am. Jour. Med. Sc.*, 1914, CXLVIII, 187) report cases of infection with the closely related *Schistosomum japonicum*; Reed, *Am. Jour. Trop. Dis. and Prev. Med.*, 1915, III, 247; Mann, *Jour. A. M. A.*, 1916, LXVII, 1366; Ferguson, *Jour. Royal Army Med. Corps*, 1917, XXIX, 57; Leiper, *Jour. Am. Med. Assoc.* 1920, LXXV, 761.

are obtainable. The bacteriological study of the blood requires not only a thorough working-knowledge of the principles underlying the bacteriological technic but, in many cases, special training, also, in the isolation and differentiation of the suspected organism.¹

Technic.

The success of this work depends partly upon the obtaining of a sufficient quantity of blood and partly upon the selection of the culture-media for the future development of the organism present. The amount of blood required varies from 5 to 20 c.c., so that it must be obtained by venous puncture. The vein usually selected is the median basilic or the median cephalic at the bend of the elbow, but, if these be not available, the veins of the dorsum of the hand may be used, although the latter are less satisfactory. The site of puncture should be carefully cleansed, using all the precautions taken for any surgical procedure. As a rule, contaminations from the skin are rare providing this cleansing has been adequate. In some cases, especially in very obese patients, it is necessary to expose the vein by incision of the skin and subcutaneous fat over the vein.

The instrument best adapted for venous puncture is, in the writer's opinion, the Lürer syringe, which is made entirely of glass and has a tightly fitting platinum needle.² Sterilization is best done by the use of dry heat to 150°C. for one hour, boiling in 1 per cent. sodium carbonate solution for 45 minutes, or using steam under pressure in an autoclave for 15 minutes. These instruments should never be sterilized with carbolic acid or bichlorid solution owing to the danger of inhibiting the growth of the suspected organisms. It is well, also, to sterilize a pair of forceps to use in fitting the needle on the syringe. The properly sterilized outfits should be kept in long test-tubes, plugged with cotton, the point of the needle being protected by resting upon a pad of sterilized cotton.

When the above precautions have been taken, an elastic band or a towel may be fastened about the arm above the point of puncture, to produce constriction of the vessels and distention at the point of puncture. The skin may be anesthetized with a spray of ethyl chlorid, if the patient be very susceptible to the slight pain caused by the puncture. The needle is plunged into the vein against the direction of the blood current, care being taken that the point of the needle is sharp as, otherwise, the vein may roll about beneath it. Very slight aspiration is necessary to draw the blood into the syringe, as the blood tends to flow into the instrument through its own force. The usual amount withdrawn is 10 c.c. but occasionally 20 c.c. are preferable, especially if the patient is plethoric and can stand the loss easily. When the desired amount is obtained, the bandage is removed before the needle is withdrawn.

After withdrawal from the vein, the needle is quickly removed from the syringe and the blood divided among several tubes containing agar melted and cooled to 40°C., the tip of the syringe being passed through the flame

¹ See Cummer, *Ohio State Med. Jour.*, 1914, X, 201; Lintz, *New York Med. Jour.*, 1915, CI, 660; McLeod and Bevan-Brown, *Jour. Path. and Bact.*, 1918, XXII, 76.

² See Judd and Simon (*Jour. Am. Med. Assn.*, 1915, LXIV, 822) for the use of the Keidel tube charged with culture medium.

before inoculating each tube. In deciding as to the amount of blood to add to the agar, a general rule is to increase the amount of blood when the feeble-growing organisms, such as the pneumococcus and gonococcus, are suspected. The usual proportions are 2 to 3 of blood to 5 of agar. It is, probably, preferable to add the blood to fluid media, such as bouillon or litmus-milk, using 1 to 2 c.c. of blood to 100 c.c. of media in order to overcome the normal bactericidal effects of the blood.¹ The blood and fluid media are then directly incubated at 37°C. In the case of the agar tubes, the blood and media are mixed by shaking as quickly as possible, poured into Petri dishes and placed in the incubator. It is advisable to use both solid and fluid media. Anaerobic cultures are prepared as usual, using, preferably, a combination of a vacuum with alkalized pyrogallic acid solution to insure absence of oxygen.

If, after incubation for 24 hours, the plates show only a few surface colonies, contamination is to be considered, while if the colonies be deep and are observed in several plates, contamination may be ruled out. This must not be interpreted to mean that any colonies will, necessarily, develop within 24 hours, as the plates may have to be kept for 5 to 10 days before a negative result may be certain. However, the contaminating organisms usually show up within 24 hours. The fluid media will become cloudy if any organisms are present. From the plate colonies and the fluid media sub-cultures are then made upon different media and every expedient possible used to isolate and identify the offending organism. Usually the infecting organism may be obtained in pure culture as a mixed infection in the blood is very uncommon.²

Organisms Found in the Blood.

1. *Bacillus Typhosus*.

Instead of transferring the blood, withdrawn from the vein, to bouillon or melted agar, one may add it directly to sterilized ox-bile in the proportion of 1 or 2 c.c. of blood to 5 of the bile. The blood may, if desired, be obtained in this case from a puncture of the ear (the puncture being preferably made through a coating of collodion and the blood allowed to drop directly into the tube of bile). The mixture of bile and blood is allowed to incubate at 37°C. for 12 to 15 hours, when transfers of a few loopsful are made to the Drigalski-Conradi or modified Endo media discussed under Feces. The characteristic colonies are then sub-cultured and the agglutination tests made as described in the next section.

According to Kolle and Hetsch,³ this bacillus of Eberth-Gaffky shows the following characteristics: it is an actively motile bacillus with numerous flagella, stains with the ordinary dyes but is Gram-negative, produces no

¹ Hiss and Zinsser (Text-book of Bacteriology, p. 126) recommend the addition of a small piece of calcium carbonate to the liquid media in order to neutralize any free acid formed in case the pneumococcus is suspected. See, also, Gillespie (Jour. Exper. Med., 1913, XVIII, 584) for comparative viability on fluid and solid media.

² Warren and Herrick, Am. Jour. Med. Sc., 1916, CLI, 556; Bloom, Am. Jour. Dis. Child., 1917, XIII, 128; Wurtz and Sappington, Jour. Med. Res., 1918, XXXVIII, 371; Richey and Goehring, Ibid., 421; Chamberlin and Minaker, Jour. A. M. A., 1919, LXXII, 1073.

³ Die Experimentelle Bakteriologie. Berlin, 1911; Demolon, Paris Med., 1916, VI, 354; Vaughan, Jour. Lab. and Clin. Med., 1919, IV, 640.

indol in bouillon (colon bacillus does), produces no gas in glucose-bouillon (colon bacillus does), produces no acid nor any coagulation in litmus-milk (colon bacillus produces much acid, while the bacillus *fæcalis alcaligenes* is a strong alkali-former), produces no change in neutral red-agar (colon bacillus and paratyphoid bacillus B cause fluorescence of the solution and gas formation, while the bacillus *fæcalis alcaligenes* and the dysentery bacillus produce no effects), produces dew-drop colonies on litmus-lactose-agar with no change in color of the media (colon bacillus produces marked red coloration), produces acid and coagulation of the casein in litmus-nutrose-glucose solution, while no such effects are observed in litmus-nutrose-lactose solution, shows characteristic agglutination with pure culture of typhoid bacillus.

Ordinarily the agglutination tests are all that are necessary after the organism has been grown on the Drigalski-Conradi or other media. However, it is to be remembered that the freshly isolated organisms show less tendency to agglutinate than do the organisms which have been grown for some time or have been transferred through several generations.

The importance of blood cultures¹ in cases of typhoid fever may be estimated by the experimental evidence that 70 to 80 per cent. of cases are positive, oftentimes many days before the agglutination tests are positive. It has been further shown that the organisms tend to diminish in the blood during the course of the disease until they practically disappear from the blood about the end of the third week.

2. *Bacillus Paratyphosus.*

In certain cases, which clinically closely resemble typhoid fever, although running a somewhat milder course, organisms may be isolated from the blood, which are intermediate between the true bacillus typhosus and the bacillus coli communis. The clinical differentiation of these cases is often difficult, although the sudden onset of vomiting, diarrhea, chill, and fever of paratyphoid are somewhat different from the more gradual onset and step-like increase of temperature in typhoid; while the temperature-curve, the somewhat different early characteristics of the feces, the early appearance of herpes, the usual lack of splenic enlargement, etc., may serve as differential points in favor of paratyphoid.² Aside from this type, we find sporadic or epidemic cases in which the symptomatology is that of a severe gastroenteritis, the so-called "infectious meat-poisoning" usually classed as ptomaine poisoning, which is due to the bacillus paratyphosus. These organisms are widely spread in the animal kingdom and may be found even as a contamination in water supplies. Not infrequently it is found in man, as a pure saprophyte, no

¹ This work is fast becoming an absolute necessity, as the employment of the preventive inoculation for typhoid practically destroys the reliability of the Widal test of the serum of those previously inoculated. See Moreschi, *Ztschr. f. Immunitätsforsch.*, 1914, XXI, 410; McIntosh and McQueen, *Jour. Hyg.*, 1914, XIII, 409; Saski, *Ztschr. f. klin. Med.*, 1914, LXXX, 79; Carnot and Weill-Halle, *Presse méd.*, 1915, XXIII, 89; Hohlweg, *Münch. med. Wchnschr.*, 1915, LXII, 538; Lipp, *Ibid.*, 539; Swartz, *Hygiea*, 1921, LXXXIII, 852.

² See Hunt, *Arch. Int. Med.*, 1913, XII, 64; Heigel, *Wien. klin. Wchnschr.*, 1915, XXVIII, 57; Dreyer, Walker and Gibson, *Lancet*, 1915, I, 324 and 643; Irons and Jordan, *Jour. Infect. Dis.*, 1915, XVII, 234; Robinson, *Jour. Med. Research*, 1915, XXXII, 399; Bernstein and Fish, *Jour. A. M. A.*, 1916, LXVI, 167. Fond and Walker, *Pub. Health Rep.*, 1921, XXXVI, 2095) report an epidemic traceable to infection with paratyphoid B carried in head cheese.

clinical symptoms traceable to its presence being observed. It may be present in the pus of otitis media, orchitis, cholecystitis, osteochondritis, arthritis, lymphadenitis, also, as a secondary invader, in scarlet fever, measles, pneumonia, meningitis, etc.

The examination of the blood is made as previously described, transfers and sub-cultures being made upon the various media. These paratyphoid bacilli are, apparently, of two types, known as "A" and "B." Both types differ from the bacillus typhosus in that they produce gas in glucose media and show different agglutination reactions. Type A behaves in most other respects like the typhoid bacillus, except that the former causes fluorescence in neutral-red agar. Type B is differentiated from the typhoid bacillus and from type A of the paratyphoid bacillus by not coagulating litmus-milk but in producing acid (red coloration) and, after 5 to 10 days, in causing a conversion of this red color to a deep blue (due to alkali formation). The typhoid, colon, paratyphoid A and dysentery bacilli never cause a blue coloration of the milk, while the blue color due to the bacillus *fæcalis alcaligenes* is produced at once. Jordan¹ has shown that there are cultural differences between the Paratyphoid A and B strains and those of the enteritidis group, these being manifest in the formation or absence of formation of acid and gas in xylose, arabinose and dulcitol, and in the degree of rapidity of alkali formation in litmus milk. The paratyphoid A strains, which are derived from human sources, produce alkali in litmus milk much more slowly than the others and do not ferment xylose. The paratyphoid B strains ferment xylose, arabinose and dulcitol rapidly and produce alkali quickly in litmus milk. The enteritidis strains are indistinguishable from the paratyphoid strains B, but have distinct agglutinative reactions. The peculiarities of the agglutination reaction with this organism will be discussed in the next section.

3. *Bacillus Coli Communis*.

This organism is, probably, not a unitary organism, as many types of the species exist. The colon bacillus is found in all warm-blooded animals and in many cold-blooded ones as a normal habitant of the intestinal canal. These bacilli show a marked antagonism for the putrefactive bacteria, so that physiologically, they should be regarded as conservative organisms. Owing to the wide occurrence of this organism, it is frequently found as a contamination of food and water supplies unless special precautions are taken to exclude it.

Although ordinarily a harmless type, yet, from the pathologic standpoint, the colon bacillus is generally regarded as a pathogenic organism under

¹ Jour. Infect. Dis., 1917, XX, 457. See, also, Krumwiede, Pratt, and Kohn, Jour. Med. Res., 1916, XXXIV, 355; Ibid., XXXV, 55 and 357; Weiss and Rice, Ibid., 403; Krumwiede and Kohn, Ibid., 1917, XXXVI, 509; Labbé and Canat, Ann. de Méd., 1917, IV, 1; Jordan and Victorson, Jour. Inf. Dis., 1917, XXI, 554; Jordan, Ibid., 1918, XXII, 252 and 511; Jordan and Irons, Ibid., 1918, XXIII, 537; Krumwiede, Kohn and Valentine, Jour. Med. Res., 1918, XXXVIII, 80; Ibid., 1919, XXXIX, 449; Mulsoy, Jour. Inf. Dis., 1919, XXV, 135; Spray, Ibid., 1920, XXVI, 340; Jordan, Ibid., 427; Fred and Peterson, Ibid., XXVII, 539; Sartory, Prog. Med., 1920, XXXV, 95; Goeckel, Jour. Lab. & Clin. Med., 1920, V, 255; Ibid., 1921, VI, 335; Morgan, Bull. Johns Hopk. Hosp., 1921, XXXII, 195; Koser, Jour. Inf. Dis., 1921, XXIX, 67; Krumwiede, Provost and Cooper, Jour. Med. Res., 1922, XLIII, 53; Kendall and Hauer, Jour. Inf. Dis., 1922, XXX, 233 and 236.

certain conditions. In this connection we must distinguish an endogenous from the exogenous colon infections. The first are found in cases of wound infection and, more especially, in infections of the lower urinary tract into which the bacilli have gained entrance through the urethra; while the latter are observed under the following conditions. So long as the natural protective and bactericidal agents of the body are intact and so long as the intestinal epithelium is unimpaired, the colon bacilli do not pass into the organs or serous cavities of the body. However, in the course of any disease, such as typhoid, cholera and severe non-specific enteritis, in which the epithelium is destroyed, the colon bacillus may pass from the intestinal tract, either by direct contiguity of tissue or through the blood current, and assume a pathogenic rôle as a secondary invader. Moreover, should the patient be suffering with a chronic wasting disease which lowers the general resistance to infection, the continuity of the epithelium may still be preserved and the bacillus become pathogenic in many organs. Thus we find infections due to the bacillus coli communis in the kidney, urinary bladder, gall-bladder, peritoneal cavity, ovary, uterus, and other organs.¹ Pus pockets, which may have caused little clinical disturbance, may spread, become chronic and induce a septicemia, of which the etiologic factor may be determined only by resort to blood culture.

The biologic properties distinguishing the colon bacillus from the typhoid and paratyphoid bacilli are as follows: The colon bacillus is very little, if at all, motile; it produces gas in glucose media and indol in bouillon or peptone media; it coagulates milk and produces acid with red coloration in litmus milk; its colonies on litmus-lactose agar are deep-red in color (the color diffusing into the surrounding media), while those of typhoid and paratyphoid are colorless; in neutral-red agar it causes fluorescence and marked gas production, while the typhoid bacillus produces no change in the media; it does not show characteristic agglutination reactions, while the typhoid bacillus does

4. *Pneumococcus*.

This organism may be recovered from the blood in practically all cases of lobar pneumonia due to infection with this type, although it must be remembered that other organisms may be the etiologic factor in some cases. At times the pneumococcus may be obtained as early as 12 hours after the initial chill, while in other cases it may appear as late as 48 hours after the crisis. Rosenow obtained positive results in 160 out of 175 cases, Prochaska 48 out of 50, while Cole and Lenhartz report only 30 per cent. of positive findings.² The finding of the pneumococcus in the blood does not, necessarily, indicate an unfavorable prognosis, although a large percentage, 40 to 50, of the cases in which it is found result fatally.³ The earlier the organisms are found the more grave the prognosis. The number of organisms found by culture is not as large as in other types of blood infection, so that Fraenkel is led to believe that the prognosis is bad when a large number of colonies develop from 1 c.c. of blood. The infection may spread through the lymphatic channels

¹ Oliver and Schwab, *Jour. Inf. Dis.*, 1920, XXVI, 336, report the isolation of a bacillus of the colon group from cultures of the abscesses in a case of furunculosis.

² See Lyall, *Jour. Am. Med. Assn.*, 1912, LVIII, 1841.

³ See Hastings and Boehm, *Jour. Exper. Med.*, 1913, XVII, 239.

to the blood, so that we may find these organisms in the bile, urine, milk and other body secretions. Moreover, we may have many infections in other organs as a complication of the original pneumonia.

Oftentimes, we find the pneumococcus as the cause of primary pathological conditions in many organs, when there has been no antecedent pneumonia. Thus we may have a pneumococcic otitis media, bronchitis, pleuritis, peritonitis, meningitis, endocarditis, pericarditis, orchitis, tonsillitis, cystitis, salpingitis, enteritis, conjunctivitis and keratitis. The causative factor may be discovered only by examinations of the various excretions related to the special condition. While the blood culture may be of little clinical value in the diagnosis of lobar pneumonia as the clinical findings are usually so clear-cut, yet it may be the only means at our disposal in solving the mystery of an obscure infection in other parts of the body, as for instance in a malignant endocarditis.¹

The blood is taken as previously outlined and is transferred to agar, blood-agar, plain, serum or ascitic bouillon, or litmus-milk. The organisms which are especially confusing from the standpoint of differentiation are the various types of streptococci. On blood-agar plates the pneumococcus shows an especially characteristic greenish hemolytic colony. The streptococcus viridans produces the same green zone, while the streptococcus pyogenes shows no green color but a large colorless hemolytic zone. Blood bouillon shows no hemolysis with either the pneumococcus or streptococcus viridans, while a marked burgundy-red color appears in the presence of streptococcus pyogenes. The differentiation between the pneumococcus and streptococcus viridans is made by the use of bile acids. Bouillon cultures of pneumococcus are cleared while those of the viridans are unaffected. Further, the pneumococcus ferments inulin solutions, while most strains of streptococci do not produce this result. The detection of the capsule and the agglutination reactions clear up the diagnosis.

Rosenow's Capsule Stain.

This method² is, in the writer's opinion, the best at our command for this purpose. The technic is as follows: Make a thin smear on a perfectly clean slide or cover-glass. If the material, such as sputum, is too thick, add enough distilled water so that it can be spread evenly by means of a piece of fine tissue or cigarette paper. In cases of cultures (blood-agar, serum, glucose or Loeffler's blood-serum being preferable), remove a small amount of the growth from the surface of the medium and at once mix thoroughly with a loopful of serum on the slide, or, better still, make a rather dense suspension in a few drops of distilled water and then mix an equal quantity of this suspension with serum, and spread by means of tissue paper. As the smear becomes nearly dry, cover for 10 to 20 seconds with 5 to 10 per cent. aqueous solution of tannic acid; wash in water and blot; stain with carbol (saturated alcoholic solution gentian violet, 1 part, 5 per cent. aqueous carbolic acid, 4 parts) or anilin gentian violet half a minute to a minute, heat over flame

¹ See Rosenow, Illinois Med. Jour., 1912, XXI, 425; Jour. Infect. Dis., 1912, XI, 94; also, Cole (Jour. Am. Med. Assn., 1912, LIX, 693), for a discussion of immunity in pneumonia; Cole, Arch. Int. Med., 1914, XIV, 56; Day, Ill. Med. Jour., 1916, XXIX, 354.

² Jour. Infect. Dis., 1911, IX, 1.

but do not boil; wash in water again; Gram's iodine solution for $\frac{1}{2}$ to 1 minute; decolorize in alcohol (95 per cent.); stain for 2 to 10 seconds, depending on the thickness of the smears, with saturated alcoholic (60 per cent.) solution of Grübner's eosin; wash in water and blot finally, clear in xylol and mount in balsam or examine directly. If the organism, like the *Bacillus mucosus*, is Gram-negative, the bacillus may be stained with Loeffler's or aqueous methylene blue.

The pneumococci are stained deeply brownish-black, sharply differentiated from the capsule, which is stained pink. Beautiful results are also obtained with the streptococcus mucosus. In the thickest part of the smear the space occupied by the capsule may be perfectly clear; elsewhere in the smear, if properly made, where the conditions are suitable for absorption of eosin, the capsule is stained deeply pink; not rarely a clear refraction zone (often mistaken for the capsule in former methods) may be seen peripherally to a distinctly stained, often large capsule. In case of sputum in which the cocci are embedded in a more or less tenacious mucus, the capsules, at times, are not rendered stainable by the above method. In that case it is well to fix and stain simultaneously with the 2 per cent. aqueous tannic acid, 4 parts, and saturated solution of gentian violet, 1 part. This modification often gives beautiful results. The cocci, however, decolorize easily and the tannic acid-gentian-violet may be followed by carbol-gentian-violet and then the usual procedure. Ordinary carbol-fuchsin, diluted five to ten times, and aqueous eosin (50 per cent. saturated solution) may also be used to stain the capsule, although the saturated alcoholic (60 per cent.) eosin has given the best results. Decolorization after the modified Gram procedure of tannic acid-fixed smears is more rapid than in the case of heat-fixed smears, which fact should be borne in mind.

By the use of this method it has been determined that the capsule of the pneumococcus and allied organisms is not difficult to preserve or readily soluble in water, as is generally believed. To stain the capsule is a problem of rendering it stainable rather than one of preservation. The reactions which accomplish this appear to be colloidal reactions.

5. Streptococci.

In general infections with any of the varieties of the streptococcus, blood cultures reveal the offending organism in a large percentage of cases, depending, of course, upon the location and severity of the process. There are many varieties of streptococci, of which some are saprophytic and some pathogenic. Pathologically, streptococci are found in the blood in many conditions. They may obtain entrance to the blood by rupture of a streptococcic abscess into a vessel. Infected thrombi, as met with in puerperal sepsis, may form the source of the invasion. Long-standing infections, such as empyemas, may so lower the resistance of the patient that a septicemia may result. An especial point of invasion is through wounds, the infection here often being extremely dangerous. A further type is the cryptogenetic septicopyemia in which the source of infection can not be traced.

The organism may be obtained from the blood in practically all cases of mycotic endocarditis due to this streptococcus. In puerperal sepsis the results are not so frequent, only about one-fourth of the cases being positive. The cryptogenetic types associated with metastatic lesions in the joints and organs show a much larger percentage of positive findings. In scarlet fever we find, according to Hektoen, that streptococci may occasionally be found in the blood in cases which run a short, mild, and uncomplicated course; they occur with relatively greater frequency in the more severe and protracted cases in which there may develop local complications and signs of general infection, although they may not be demonstrable in fatal cases. The streptococci may also be found in diphtheria, measles and small-pox especially in the fatal cases, in which they act as secondary invaders. It is to be emphasized that the prognosis is not, necessarily, bad when streptococci are found in the blood, although there is a large mortality.

The morphology of these organisms is variable and the varieties numerous.¹ The pathogenic types, isolated directly from pathologic lesions, appear to consist of rather long chains of more than 8 pairs of cocci when grown in bouillon, while the non-pathogenic types form shorter chains. Involution forms are common in specimens from culture. Some of them are encapsulated, two organisms being enclosed. This may lead to confusion with the pneumococcus but the differential points given under the pneumococcus will serve to distinguish them. Usually the microscopic examination of the stained specimen will be all that is necessary, but occasionally careful work is needed to identify them. In doubtful cases, $\frac{1}{2}$ to 1 c.c. of the blood withdrawn by venous puncture may be injected intraperitoneally into a mouse, when a general streptococcic septicemia will ensue.

Many other organisms have been and may be isolated from the blood. Thus the staphylococci are not infrequently the cause of a bacteriemia arising from an endocarditis, osteomyelitis, or furunculosis. The gonococcus is often found in cases associated with gonorrheal endocarditis, arthritis, parotitis, myositis, etc. The meningococcus, the influenza bacillus, bacillus mucosus, bacillus of anthrax and of glanders have been obtained from the blood in suitable cases.² The tubercle bacillus has been repeatedly found in cases of both acute and chronic tuberculosis. However, much recent work since the publication of Rosenberger, who attempted to prove that tubercle

¹ See Winslow, *Jour. Infect. Dis.*, 1912, X, 285; Jungmann, *Deutsch. Arch. f. klin. Med.*, 1912, CVI, 283; and Bergey, *Jour. Med. Research*, 1912, XXVII, 67. See p. 54 for a discussion of streptococcic sore-throat. Also, Thalheimer and Rothschild, *Jour. Exper. Med.*, 1914, XIX, 429 and 444; Simons, *Quart. Jour. Med.*, 1914, VII, 291; Rosenow, *Jour. Am. Med. Assn.*, 1914, LXIII, 903; Oille, Graham and Detweiler, *Ibid.*, 1915, LXV, 1159; Rosenow, *Jour. A. M. A.*, 1915, LXV, 1687; Lintz, *Jour. Lab. and Clin. Med.*, 1918, III, 509.

² Tunncliffe (*Jour. A. M. A.*, 1917, LXVIII, 1028) has isolated from the blood in pre-eruptive and eruptive stages of measles, by using anaerobic cultures, a small, round, sometimes flattened diplococcus, which may appear in short chains, sometimes as clumps of cocci of varying sizes. Reed (*Jour. A. M. A.*, 1914, LXIV, 1047; *Ibid.*, 1916, LXVI, 336) announced the finding in the blood of a coccus, the epilepticoccus which he believed to be the cause of epilepsy. Later he (*Ibid.*, 1907) described a large spore-bearing organism styled bacillus epilepticus as the etiologic factor. Wherry and Oliver, *Ibid.*, 1916, LXVII, 1087) identify this organism as the bacillus subtilis. See, also Caro and Thom, *Ibid.*, 1908; Terhune, *Ibid.*, 1155.

bacilli were present in the circulating blood of incipient tuberculosis and that a primary bacteriemia was always present in these cases, has absolutely refuted this finding and has emphasized the importance of eliminating the possibility of the presence of acid-fast bacilli in the reagents and, even, in the distilled water used.¹

It is probably true that the organism at the bottom of every infectious disease of known origin may be obtained from the blood at some stage of the infection. It is important, therefore, that the clinical examination include blood cultures in any case of obscure origin, as in this way treatment and prognosis will be markedly influenced. Churchill and Clark² have recently called attention to the importance of this work in children.

VII. SERUM PATHOLOGY

This section of hematology is very closely associated with pathology and biochemistry and can, therefore, be taken up only in brief outline.³ As a matter of fact, changes in the number of the cells as well as in the percentage of hemoglobin must be dependent, to a certain extent, upon the more obscure changes which are taking place in the plasma in various diseases. Our ability to fathom the secrets of the many physical and chemical changes of the plasma has been so slight that we have hitherto neglected to take into consideration anything but the changes in the cells, which can be so easily studied by the various methods previously outlined. Our knowledge of the various types of immunity to infection and of the many factors concerned therein has increased to such an extent in recent years that a complete discussion is out of place here. By the elaboration of the side-chain theory of Ehrlich and of the opsonic theory of Wright, we have come somewhat nearer to a proper realization of the importance of the serum in all infections as well as in many diseases in which great metabolic disturbance is evident. For that matter one can hardly imagine a condition in which the blood plasma may not show some characteristic change, inasmuch as the nutrition of the entire body can come only through the blood. When one considers the close correlation of the various organs he may see at once that pathological changes in any of the viscera may result in an abnormal blood, which may show no variations at present capable of detection. For these reasons one hails with delight any advance in serum pathology and could but wish that his knowledge might more rapidly increase.

¹ See Berry, *Jour. Infect. Dis.*, 1914, XIV, 162; also, Rautenberg, *Berl. klin. Wchnschr.*, 1914, LI, 348 and 402; Baetge, *Deutsch. med. Wchnschr.*, 1914, XL, 591; Faber, *Jour. Am. Med. Assn.*, 1914, LXIII, 1656; Jousset, *Bull. l'Acad. de Med.*, 1915, LXXIII, 203; Luciarini, *Rif. Med.*, 1915, XXX, 253 and 281; Austrian and Hamman, *Bull. Johns Hopkins Hosp.*, 1915, XXVI, 293; Kessel, *Am. Jour. Med. Sc.*, 1915, CL, 377; Honeij, *Jour. Infect. Dis.*, 1915, XVII, 376; Wilson, *Jour. Infect. Dis.*, 1916, XIX, 260; Hall and Harvey, *Jour. Med. Res.*, 1917, XXXV, 265; Distaso, *Tubercle*, 1921, II, 251.

² *Am. Jour. Dis. Child.*, 1911, I, 193.

³ See Kraus and Levaditi, *Handbuch der Technik und Methodik der Immunitätsforschung*, Jena, 1909-1911; Citron, *Die Methoden der Immunodiagnostik und Immunotherapie*, Leipzig, 1912; Dieudonné, *Immunität, Schutzimpfung und Serumtherapie*, Leipzig, 1913; Vaughan, *Protein Split Products in Relation to Immunity and Disease*, Philadelphia, 1913; Kolmer, *Infection, Immunity and Specific Therapy*, Philadelphia, 1915; Wells, *Chemical Pathology*, Philadelphia, 1920.

Ehrlich's Side-chain Theory.¹

The early work of Ehrlich, published in 1885, advanced a theory to account for various phases of immunity, especially of the action of the blood in producing antitoxins against various poisons elaborated by infectious agents. It is to be said that no such formation of antitoxin against the ordinary medicinal poisons has been found.

According to this theory, the protoplasm of the cell consists of a central group of molecules (*Leistungskern*), in which the inherent vital characteristics of the cell are located and whose integrity is necessary for normal cell life. At different portions of the cell certain other molecular groups are attached exactly as side-chain groups are attached to the benzene nucleus of organic chemistry. These groups or, as Ehrlich styles them, side-chains are capable of uniting with various material which is brought into intimate relationship with the cell structure. Such materials are foods, toxins, and other injurious

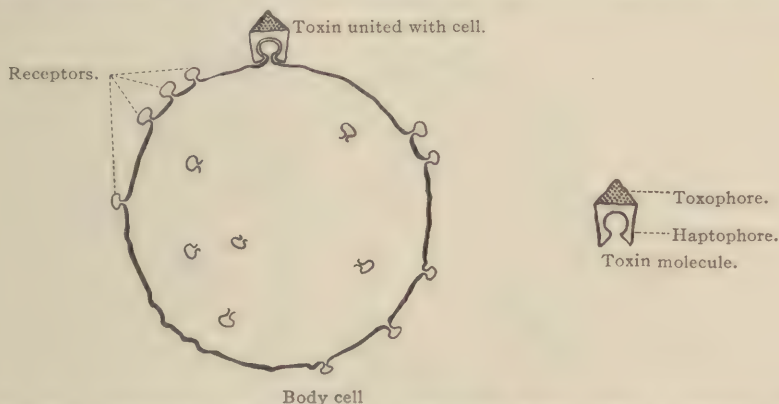


FIG. 152.—Illustrating the mechanism of the toxin-cell union by the intermediation of receptors. (*Da Costa.*)

agents. In order that foods may be taken up by the cell it must possess certain groups which will enable it to combine with the groups in the side-chain of the cell. It must be, in other words, homologous or, as Ehrlich states, must bear the same relationship to the side-chain which Fischer has applied in his assumption of the "key in the lock" hypothesis regarding ferment action upon the various types of hexoses. In the nomenclature of Ehrlich the side-chains are styled *receptors* and the group of the food or of the toxin which combines with these receptors is known as the *haptophore* group. These receptors, as well as haptophores, possess specific affinity, uniting with one another only when homologous.

It has been found that a toxin molecule has certain injurious effects upon the cell; it is, therefore, necessary to ascribe this action to other than the two groups above mentioned, as the union of a haptophore with a receptor

¹ In this connection see Sachs, *Die Haemolysine*, Wiesbaden, 1902; Kraus and Levaditi, *Handb. d. Tech. u. Meth. d. Immunitätsforschung*, Jena, 1907-1911; Adami, *Principles of Pathology*, New York, 1910; Dieudonné, *Immunität, Schutzimpfung und Serumtherapie*, Leipzig, 1913; Kolle and Wassermann, *Handbuch der pathog. Mikroorg.*, Jena, 1912; Kolle and Hetsch, *Die Experimentelle Bakteriologie*, Berlin, 1911.

would form an inert substance. Ehrlich, therefore, assumes the presence in the toxin¹ molecule of a second group which he styles the *toxophore* group, which exerts the untoward effect upon the cell. The toxophore group in itself can unite with the cell only through the medium of its haptophore group. As the cell becomes irritated by the presence of the toxophore group, it endeavors to overcome this by a new formation of receptors. According to the strength of the irritation, many more receptors will be found that can combine with the toxin material present, so that many of the extra receptors pass out into the circulation in the form of free receptors. This is a graphic explanation of the fact that the cell when irritated by toxic material elaborates substances from its own protoplasm, which have a neutralizing effect upon the toxic substance. These free receptors (haptines) form the antitoxins. They combine only with homologous toxin material and are, therefore, specific. Welch believes that these antitoxins have a second function



FIG. 153.—Illustrating the elaboration and action of antitoxin. (Da Costa.)

beside that of neutralization of toxin, namely, an irritating one upon the bacterial invaders so that these organisms are forced to elaborate similar substances for their own protection. Toxins which have been deprived of their toxophore group are known as *toxoids* and can combine with the receptors of the cell, exerting no untoward effect upon the cell. These toxoids may also unite with antitoxin through the medium of their haptophore group. Occasionally toxins are incompletely combined with the antitoxins; that is, the antitoxic material is not sufficient in amount completely to neutralize the toxin, so that such toxins may still combine with the cells and exert a modified poisonous effect. Such attenuated toxins are known as *toxones*.

It has been found that the injection into animals of bacteria, various body cells, and certain secretions of some animals, as for instance, snake venom, gives rise to the development of specific antibodies in the blood serum of the animal so treated, the substances injected being named antigens.²

¹ See Coca, Jour. Infect. Dis., 1915, XVII, 351.

² See Pick (in Kolle and Wassermann's Handb. d. pathogen. Mikroorg., 1912, I, 685) who shows that true antigens are always albumin-containing colloids. Also, Elliott, Jour. Infect. Dis., 1914, XV, 501; Salus, Biochem. Ztschr., 1914, LXVII, 357; Porter, Biochem. Jour., 1915, IX, 1; Banzhaf, Sugiura and Falk, Jour. Immunol., 1916, II, 125.

Such blood or serum will be found to have a lytic (destructive) action upon cells similar to those injected. Such sera are specific, that is, they act only upon the kind of cell used in the injection. The term hemolysis has been introduced to express the destructive action upon the erythrocytes shown by the dissolving out of the hemoglobin from the red cell. The stroma or discoplasm of the red cell is a membrane which shows peculiar relations to diffusion of various materials into the cell and to the passage of hemoglobin and other cellular material from the cell. Its chief function seems to be to prevent, as far as possible, any loss in hemoglobin. If this membrane becomes permeable, then we must assume the action of some toxic material. The term *hemolysis* has reference merely to the abnormal loss of hemoglobin and not to any disturbance beyond increased permeability of the stroma. The stroma of these cells remains behind and may be seen in the centrifuged specimen as the so-called shadows. Hemolysis must, therefore, be considered as a sign of protoplasmic death. Substances (hemolysins) bringing about

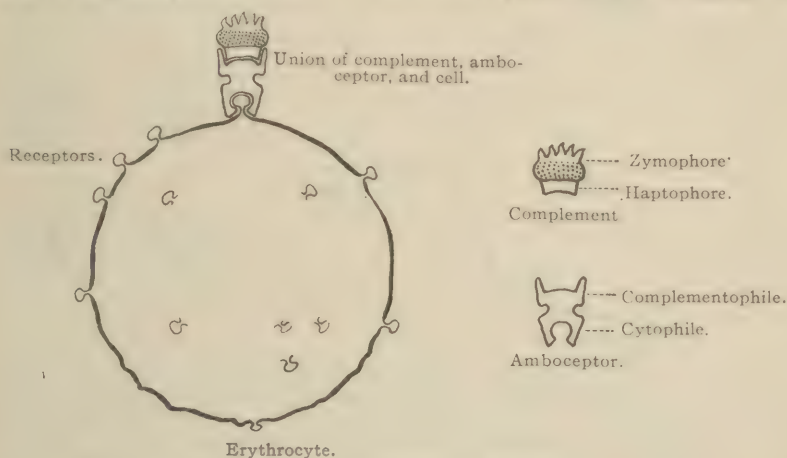


FIG. 154.—Illustrating the mechanism of hemolysis. (*Da Costa.*)

such change belong, necessarily, in the class of blood poisons. Such hemolysins are increased or lowered temperature, various inorganic compounds, such as distilled water, ammonium salts, and organic compounds, such as urea, bile acids, ether, alcohol, chloroform, solanin, saponin, and digitalin. The saponins are among our strongest hemolytic substances, acting in dilutions of 1 to 100,000. Besides these we have various secretions, such as those of the cobra, spider, and the bees which are active hemolytic agents.

It has been observed by various workers that hemolysis is prevented when the serum is heated to 60°. This points to the fact that some substance is destroyed which is of great importance in this process. In addition, it has further been found that the renewing of the activity of the old heated serum by adding a supply of fresh isologous serum will restore the hemolytic activity. It is evident, therefore, that there are two factors which must be taken into consideration, one is a thermostable (heat-resisting) substance, while the other is thermolabile (destroyed by heat). To the first of these Ehrlich has

given the name *amboceptor* and to the second the name *complement*. The amboceptor has been shown to have two haptophore groups, with one of which it unites to the receptor of the cell and with the other to the complement. The haptophore group which unites with the cell is known as the cytophile, while the one uniting with the complement is termed the complementophile. For hemolysis, therefore, we must have the cell receptor, the amboceptor, and the complement. The complement has also been shown to have two groups analogous to those of the toxin molecule. The first is the haptophore group, while the second is the zymophore group, through which the destructive action upon the cell is manifest.

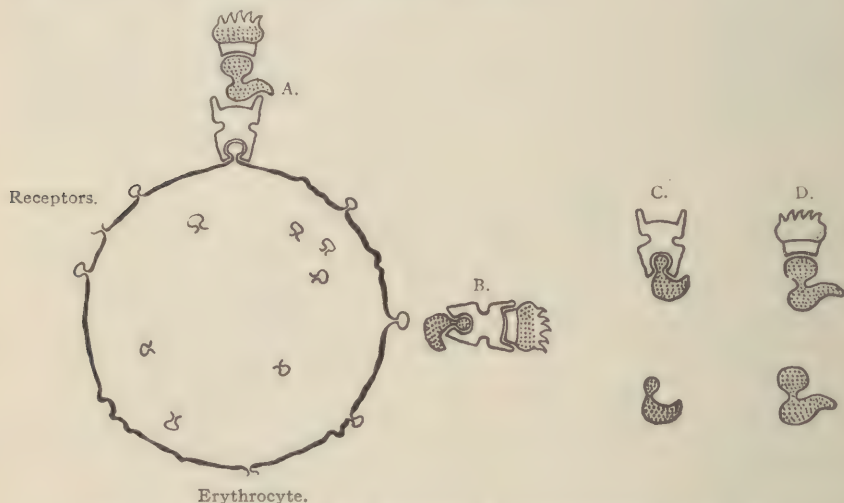


FIG. 155.—Illustrating the Mechanism of Antihemolysis. (Da Costa.)

A, Interference of anticomplement with complement-amboceptor union. B, interference of antiamboceptor with amboceptor-cell union. C, antiamboceptor-amboceptor union. D, anti-complement-complement union.

The amboceptor is formed within the body as the result of cellular hyperactivity aroused by the irritant action of the toxic material. The complement is probably derived, for the most part, from the leucocytes, and acts very much as an enzyme. It can exert its toxic action only when united with the cell by means of the amboceptor, so that free complement has no injurious effect.

It has been frequently observed that the red cells are more resistant than normally, while in many cases they appear less resistant to hemolysis. This is explained by the side-chain theory very much as it explains the formation of antitoxin. These antihemolysins are formed within the blood plasma after inoculation with hemolytic material. The hyperactivity of the cell causes it to throw off two types of such bodies, namely, *anticomplement* and *anti-amboceptors*. The former combines with the haptophore group of the complement and the latter with the cytophilic group of the amboceptor, each of these combinations making it impossible for the necessary union of cell, amboceptor, and complement to occur.

It has been found that frequently the serum of an animal, which has been injected with certain bacteria or with certain body cells, shows the peculiar property of agglutinating or clumping such bacteria or cells when these latter are added to it. This condition is known as agglutination and the agents bringing it about are styled *agglutinins*. These substances are developed in the blood of the animal during the process of adaptation toward the presence of such foreign material. Agglutinins from the standpoint of the side-chain theory are free receptors, having a haptophore group which unites with the receptor of the cell or of the bacterium, and cause agglutination through the presence of a second group known as the zymophore or agglutiniphore group. Agglutination, therefore, does not require the presence of a complement.

In some cases, intraperitoneal injection of body fluids or of solutions of certain proteins into animals brings about a condition which enables the blood serum of such animals to cause a precipitation of the protein to which the animal has been adapted. This fact has been taken advantage of in formulating a medicolegal method for the detection of blood of different animals and will be taken up in a later section. The *precipitins* consist of free receptors combining, by means of their haptophore group, with the receptor of the cell and exerting their precipitating effect through the medium of their zymophore or precipitinophore group.

Phagocytosis.

According to the theory of Metchnikoff, the leucocytes are capable of incorporating into their substance materials which are foreign to the blood in which they circulate. This process is known as phagocytosis and is one of the greatest protective measures which the system has for its fight against bacterial invasion. When the blood becomes laden with bacteria, as in the various infectious diseases, we find a leucocytosis in practically all cases, the exceptions having been previously noted. This is the natural sequence if the system is to rid itself of these invaders. The leucocytes are drawn by chemical attraction (chemotaxis) toward the bacteria and attempt to swallow them by throwing pseudopodia about them and drawing them into the protoplasm. This is successful in many cases, while in others it is not, so that the question of ascendancy of the leucocyte or of the bacterium will depend upon the degree of phagocytosis. Strangely enough much variation is shown in the susceptibility of different organisms to phagocytosis. Thus the pneumococcus at times is very difficultly amenable to phagocytosis, while at others it is easily acted upon. The recent work of Rosenow¹ has thrown much light upon the mechanism of this action.

Opsonins.

Realizing that there was a more definite basis for phagocytosis than was embraced in the older conceptions, Wright² introduced the idea of opsonins to designate the presence in the blood serum of substances which render the

¹Jour. Infect. Dis., 1906, III, 683; also, Kite and Wherry, *Ibid.*, 1915, XVI, 109; Tunnicliff, *Ibid.*, 1916, XIX, 97; Douglas, Proc. Royal Soc. London, 1916, LXXXIX, 335; Wolff, *Nederl. Tijdschr. v. Geneesk.*, 1916, II, 1789.

²Proc. Royal Soc., 1903, LXII, 357.

various bacteria subject to phagocytosis. The normal blood serum contains such opsonic material for the various bacteria with which it may be infected, but this varies greatly toward the different organisms. Thus we may find individuals showing much more opsonin (a higher opsonic index) toward one organism than toward another. This explains, in a way, the well-known fact that different people are variably susceptible to the same disease, while the same individual may be strongly resistant toward infection with one organism, but easily a victim of another infection.

Regarding their chemical nature very little is known. There seems to be a certain amount of evidence which points to the fact that these opsonins belong to the class of globulins, while Simon and Lamar¹ have shown that they are apparently associated with the euglobulin fraction. Quite as little is known of the structure of the opsonins, so that it is at present doubtful in what position to place them in the side-chain theory. According to Hektoen,² they may contain a haptophoric group which unites with the bacterial or other receptors and also an opsoniphorous group which brings about changes in the cell, making it capable of phagocytosis. According to Savtchenko and Dean, the opsonins should be regarded as amboceptors, while Greig-Smith looks upon the process of opsonification as the first stage of agglutination. All of these theories must wait for future confirmation. The opsonins are thermolabile and are usually destroyed by heating for 10 minutes to 60°C. They occur in all classes of vertebrates and show here a peculiar characteristic, namely, that the serum of different animals is capable of activating various organisms for phagocytosis by leucocytes of animals of different species. This would bring the opsonins into the same field as the agglutinins, precipitins, and hemolysins.

From a clinical standpoint, opsonins are frequently found to be diminished in certain bacterial infections. It is, therefore, conceivable that the resistance of the patient or, in other words, his phagocytic power might be increased by the addition of substances which could act as opsonic material. Such substances are the bacterial vaccines of Wright, suspensions in physiological salt solution of dead cultures of the organism to which the patient shows a diminished power of phagocytosis. The relation of the phagocytic power of the patient, as evidenced by the number of organisms which a definite number of leucocytes takes up under the opsonifying influence of this serum, as compared with the same condition in the case of the serum of a normal individual, is known as the *opsonic index* toward the organism investigated. The number of bacteria taken up by the leucocytes of the normal individual is taken as one. For preparation of these vaccines see last chapter.

According to Wright, the injection of a dose of vaccine is followed by a decrease in the opsonic index (the negative phase), which is of variable degree and duration, depending upon the dose. This negative phase is followed by an increase in the opsonic power of the leucocytes (the positive phase), which is associated with improvement in the condition of the patient. The various

¹ Bull. Johns Hopkins Hosp., 1906, XVII, 27.

² Jour. Am. Med. Assn., 1906, XLVI, 1407; Ibid., 1907, XLVIII, 1739; Illinois Med. Jour., XIII, 9; also, Zinsser and Cary, Jour. Exper. Med., 1914, XIX, 345.

doses of the vaccine should be so administered that it is never given during a negative phase. While a low opsonic index is the rule in chronic cases, high indices may be observed with active systemic manifestations of acute cases. As a rule, it is more generally beneficial to use a vaccine prepared from the discharges of the patient than it is to use an already prepared vaccine of the same organism. The reason for this is that so much difference exists in the various strains of the same organism that little or no result may follow the administration of a stock vaccine. It is not always easy to gauge the dosage of a vaccine in any particular case, as the idiosyncrasy of the patient plays such an important rôle. It is better practice, therefore, to start with a small dose and obtain no reaction than it is to give an overdose which will prove very harmful. Failure to observe this precaution is, in the writer's opinion, the reason that tuberculin so soon fell into disrepute and is now being restored to such great favor.

It has been found by various workers that the administration of a proper dose of a vaccine is followed by certain local symptoms, such as swelling, redness, and tenderness at the point of injection, while certain systemic symptoms, such as rise in temperature, general malaise, and pains in the joints are, practically always evident. It has, therefore, become the practice of many of our workers, to gauge the results of vaccine treatment more by the reaction than by the determination of the opsonic indices of their patients.

Allergic Reactions.

The vaccines discussed above are used almost entirely for therapeutic purposes. While it is highly probable that any of the vaccines might give characteristic reactions in cases infected with the same organism, yet they have not been put to extensive diagnostic use. However, certain suspensions of organisms are, at present, widely employed in this way.

It is evident, from the preceding discussion, that the serum of a patient contains bodies acting as neutralizing agents against the toxins of the infecting bacteria. This property varies with the individual and with the severity (dosage) of the infection. Patients, who have acquired immunity against certain infectious diseases, either by passing through an attack of the infection or by use of protective vaccination (small-pox or typhoid fever), or who are in the process of acquiring immunity, that is, are at the time passing through an active infection, show a certain changed reactivity to infection with the same organism. In other words such patients are not insensible to reinfection, but show a changed reactivity, as von Pirquet¹ has shown, especially as regards the time, quality and quantity of the reactions observed. This is the condition known as Allergy and is shown only by patients immune or becoming immune (infected) to the suspected organism.²

¹ Arch. Int. Med., 1911, VII, 259.

² See. Gurd, Jour. Med. Research, 1914, XXXI, 205; Meigs, Jour. Infect. Dis., 1914, XV, 541; Zinsser, Arch. Int. Med., 1915, XVI, 223. Weil, Jour. Exper. Med., 1916, XXIII, 11; Blackfan, Am. Jour. Dis. Child., 1916, XI, 441; Kolmer and Moshage, Ibid., XII, 316; Stokes, Jour. Infect. Dis., 1916, XVIII, 402 and 415; Steinfield and Kolmer, Ibid., 1917, XX, 344; Solmann and Pilcher, Jour. Pharm. and Exp. Therap., 1917, IX, 309; Kolmer, Immerman, Matsunami and Montgomery, Jour. Lab. and Clin. Med., 1917, II, 401.

Normal individuals do not show the reaction so quickly nor so intensely as do the infected or immune subjects, providing the dosage is properly regulated, as the former have sufficient antibodies to overcome the effects of the amount of toxin, which will produce local or general reactions in the infected patient.¹ These allergic phenomena are due to enzymatic preteolysis occasioned by the introduction of the foreign protein into the system and are, of course, closely related to the sensitization known as anaphylaxis. Many such reactions to food proteins are observed in certain skin diseases.²

Tuberculin Reactions.

Method of Koch.

This is the earliest method for the diagnostic use of tuberculin. The normal temperature variations of the patient are first determined for 24 hours previous to the injection. The preparation used is Koch's old tuberculin, which is a water and glycerin extract of the cultures of the tubercle bacillus, of such a strength that 1 c.c. is equivalent to 1 gram of tuberculin.

The injection may be made into the deeper tissues either in the intrascapular or gluteal region by means of a properly sterilized hypodermic syringe. The amount of tuberculin to be injected depends on the age and condition of the patient. As a rule $\frac{1}{2}$ to 1 mg. is given to a robust adult, while a weak patient or a child receives $\frac{1}{10}$ to $\frac{3}{10}$ mg. These strengths are easily made by diluting the original solution with the proper amount of $\frac{1}{2}$ per cent. carbolic acid solution. The temperature variations should then be followed, observations being made every two hours. A rise of $\frac{1}{2}^{\circ}\text{C}$. ($\frac{9}{10}^{\circ}\text{F}$.) within a few hours or even as late as 36 hours, is taken as a positive result, although some observers require a more marked rise before admitting a positive finding. Few other symptoms are noticed but, if the dose has been large, marked malaise, chill, high fever, and local reactions may obtain. If no rise in temperature is observed after the first injection, a second or a third one may be given, the dose being doubled each time but exceeding, under no circumstances, 10 mg. It is possible that a single injection of 5 or 10 mg. may give more diagnostic results than repeated doses up to this amount, although it must be admitted that such large doses may react, in rare cases, positively in normal individuals.

A positive result is very strong evidence of an active tubercular condition. A negative result is less reliable as some tubercular conditions, such as peritonitis or encapsulated foci elsewhere, may not react positively.

Method of Moro.

In this method use is made of a salve consisting of equal parts of old tuberculin and lanolin. A small bit of this salve is rubbed into the skin, preferably in the thoracic or abdominal region. After 24 to 48 hours small pale nodules, 1 to 2 mm. in diameter, are seen at the point of inoculation if the case be tubercular. A more severe reaction may be observed in the form of reddish miliary nodules. These nodules are limited to the point of application of the salve and disappear in 24 to 48 hours. Occasionally, some exudation

¹ See Hektoen, *Jour. Am. Med. Assn.*, 1912, LVIII, 1081; also, Vaughan, *Am. Jour. Med. Sc.*, 1913, CXLV, 161.

² See Strickler and Goldberg, *Jour. A. M. A.*, 1916, LXVI, 249; McBride and Schorer, *Ibid.*, 919; White, *Jour. Cutan. Dis.*, 1916, XXXIV, 57; Strickler, *N. Y. Med. Jour.*, 1916, CIV, 198.

may arise from the intense reactions, but this is very rare. This method is very simple and is very reliable.¹

Method of von Pirquet.

This method² is very widely used, especially in cases of infection in children. The inner side of the forearm is cleansed with alcohol and ether and allowed to dry. Two drops of old tuberculin are placed on the skin at a distance of 6 to 8 cm. from each other. The skin covered by these drops is then punctured by a large needle or gently scarified. The tuberculin is allowed to remain for 10 to 15 minutes, when the clothing may be replaced. It is wise to make a third puncture or scarification between the two tuberculin points as a control upon the patient's reaction, no tuberculin being used here. A slight swelling is usually produced which becomes surrounded by a light reddish border in a few hours, and disappears in 24 hours. If the reaction be positive, the area of puncture swells within 24 to 48 hours to a papule, reddish in color and 10 to 15 mm. in diameter. The center may be pale and be surrounded with small serous points. By comparison with the normal point, a reaction may be more clearly demonstrated. The swelling and redness begin to disappear within 48 hours, a slight pigmentation usually following this positive reaction. No general disturbance is noted as a rule. If the reaction be repeated, a marked positive result may be observed as an evidence of hypersensibility, the so-called anaphylactic reaction. This may, also, be noticed in the other methods, even though a much smaller dose be used than in the initial application.

Method of Calmette.

This method was advanced almost simultaneously by Calmette and Wolff-Eisner. One or two drops of a 1 to 100 dilution of old tuberculin in physiologic salt solution are placed in the conjunctival sac, the lids being held apart for a few seconds to permit of absorption of the tuberculin. Only one eye is thus treated, the other being used as the control. A positive reaction appears in 6 to 24 hours and may assume one of the following types: (1) Mere reddening of the caruncle and the inner surface of the lower lid; (2) the bulbar conjunctiva may take part in the process; (3) suppurative conjunctivitis with marked injection of the palpebral conjunctiva; (4) hemorrhagic conjunctivitis of the palpebral and bulbar portions with profuse fibrino-purulent exudation. An associated rise in temperature may be observed in the severe types.³

¹ See Krumbhaar and Musser, *Am. Jour. Med. Sc.*, 1914, CXLVII, 540.

² See Wachenheim, *Am. Jour. Dis. Child.*, 1912, IV, 27; Austrian, *Jour. Exper. Med.*, 1912, XV, 149; Rosenberg, *Ztschr. f. exper. Path. u. Therap.*, 1913, XII, 549; and Wachenheim, *Am. Jour. Dis. Child.*, 1913, V, 466; Klopstock, *Ztschr. f. exper. Path. u. Therap.*, 1914, XV, 13; Monrad, *Ugesk. f. Laeger*, 1914, LXXXVI, 1419; Küchenhoff, *Deutsch. med. Wchnschr.*, 1914, XL, 229; Salvetti, *Pediatria*, 1915, XXIII, 35; Fishberg, *Arch. Pediat.*, 1915, XXXII, 20; *Am. Jour. Obs.*, 1915, LXXI, 361; Kraemer, *Münch. med. Wchnschr.*, 1915, LXII, 5 and 46; Frazer, *Med. Record*, 1915, LXXXVII, 57; Palmer, *Jour. A. M. A.*, 1915, LXIV, 1312; Cattermole, *Ibid.*, 1915, LXV, 782; Manning and Knott, *Am. Jour. Dis. Child.*, 1915, X, 354; Heise, *Am. Jour. Med. Sc.*, 1916, CLI, 862; Lindberg, *Hygiea*, 1916, LXXVIII, 1553; Permin, *Hospitaltid.*, 1916, LIX, 697; Weith, *Rev. Med. de la Suisse Rom.*, 1916, XXXVI, 537; Craig, *Jour. A. M. A.*, 1916, LXVII, 1227; Weil, *Ibid.*, 1917, LXVIII, 972; Cohen, *Jour. Infect. Dis.*, 1917, XX, 233; Blechmann, *Ann. de Med.*, 1919, VI, 200; Seligmann and Klopstock, *Ztschr. f. Immunität*, 1919, XXVIII, 454; Mioche, *Nourrisson*, 1920, VIII, 42; Gammons, *Jour. Am. Med. Assoc.*, 1921, LXXVI, 1396; Bardswell, *Tubercle*, 1921, II, 433; Zinsser, *Jour. Exp. Med.*, 1921, XXXIV, 495; Strickler, *Jour. Am. Med. Assoc.*, 1922, LXXVIII, 1287.

³ See Vaughan, *Jour. Am. Med. Assn.*, 1913, LXI, 1591.

Contraindications to this test are found in any inflammation of the eye or conjunctiva, in the previous treatment of the eye with tuberculin, and recent application of tuberculin elsewhere. It would not seem that this method is advisable owing to the great discomfort which may arise and, especially, as it is not without danger of severe injury to the eye.¹

Luetin Reaction.

Noguchi² has recently announced "A Cutaneous Reaction in Syphilis," which may prove of great value in the diagnosis of this condition. His material is prepared as follows: Pure cultures of the *treponema pallidum* (see p. 680) are allowed to grow for periods of 6, 12, 24, and 50 days at 37°C. under anaerobic conditions. One was cultivated in ascitic fluid containing a piece of sterile placenta, and the other in ascitic fluid agar also containing placenta. The lower portion of each solid culture was cut out and the tissue removed. These agar columns, containing large numbers of spirochetæ, were carefully ground in a sterile mortar. This paste was then gradually diluted by adding, little by little, the fluid culture, until the emulsion became perfectly liquid. This mixture was heated to 60°C. for one hour in a water-bath and 0.5 per cent. carbolic acid was added. When this mixture is examined under the dark-field microscope 40 to 100 pallidæ may be seen in each field. This suspension is called *luetin*.³

The skin of the upper arm is sterilized with alcoholic sublimate solution before the injection. The amount of luetin injected is 0.05 c.c. to 0.07 c.c. This injection is intradermic, that is, in the skin as superficially as possible. In normal cases there appears, after 24 hours, a very small erythematous area at and around the point of injection. No pain or itching is experienced. This slight reaction gradually recedes within 48 hours and leaves no induration. In certain cases the reaction may reach a stage of small papule formation after 24 or 48 hours, after which time it commences to recede.

In positive cases the following types of reaction occur:

(A) *Papular Form*.—A large, raised, reddish, indurated papule, usually 5 to 10 mm. in diameter, makes its appearance in 24 to 48 hours. The papule may be surrounded by a diffuse zone of redness and show marked telangiectasis. The dimensions and the degree of induration slowly increase during the following three or four days, after which the inflammatory processes begin to recede. The color of the papule gradually becomes dark bluish-red. The induration disappears within a week, except in certain instances in which a trace of the reaction may persist for a longer period. This latter effect is usually met with among cases of secondary syphilis under regular mercurial treatment in which there are no manifest lesions at the time of making the skin test. Cases of congenital syphilis also show this reaction.

¹ Austrian (Bull. Johns Hopkins Hosp., 1911 XXIII, 1) advocates a similar test for typhoid fever. The antigen is a "typho-protein" prepared from a mixed culture of 80 different typhoid strains. Positive results are obtained in 95 per cent. of cases of typhoid while it is negative without exception in practically all controls. Its great advantages are that it is a bedside test and is given in the early stages of the disease.

² Jour. Exper. Med., 1911, XIV, 557; Münch. med. Wehnschr., 1911, LVIII, 2372.

³ Luetin may now be obtained either from Parke, Davis and Co. or Mulford Co. See Ward, Am. Jour. Syph., 1921, V, 482.

(B) *Pustular Form*.—The beginning and course of this reaction resemble the papular form until about the fourth or fifth day, when the inflammatory processes commence to progress. The surface of the indurated round papule becomes mildly edematous, and multiple miliary vesicles occasionally form. At the same time a beginning central softening of the papule obtains. Within the next 24 hours, the papule changes into a vesicle filled at first with a semi-opaque serum that later becomes definitely purulent. Soon the pustule ruptures. The margin of the broken pustule remains indurated, while a crust quickly forms, which falls off within a few days. The induration soon disappears leaving almost no scar. This reaction was found almost constantly in tertiary types as well as in secondary or hereditary forms which had been treated with salvarsan.

(C) *Torpid Form*.—In rare instances, the injection sites fade away to almost invisible points within three or four days, so that they may be passed over as negative reactions. Sometimes these spots suddenly light up again after 10 days or so and progress to small pustular formations.

No marked constitutional symptoms have been observed after the use of the luetin. In most positive cases a slight rise in temperature took place lasting for one day.

The conclusions of Noguchi,¹ based upon a study of 400 cases, are as follows: Luetin produces a cutaneous reaction in syphilitic and parasyphilitic patients that is most constant and severe in the tertiary and hereditary affections. During the primary and secondary stages, the reaction is infrequent, and when present is of mild degree. An exception has been found in cases in which energetic treatment has been or is being carried out and in which clinical signs of syphilis are absent.² Such cases may show a severe reaction, especially those treated with salvarsan. In certain cases of old infection in which no treatment has been taken and in which no symptoms have appeared

¹ See Ziegel, Arch. Int. Med., 1912, IX, 520; Müller and Stein, Wien. klin. Wchnschr., 1913, XXVI, 408; Klausner, Ibid., 973; Noguchi, Presse méd., 1913, XXI, 757; Wolfsohn, Jour. Am. Med. Assn., 1913, LX, 1855; Kaliski, New York Med. Jour., 1913, XCVIII, 24; Schmitter, Jour. Cutan. Dis., 1913, XXXI, 549; Brown, Am. Jour. Dis. Child., 1913, VI, 171; Foster, Am. Jour. Med. Sc., 1913, CXLVI, 645; Fagioli and Fischella, Berl. klin. Wchnschr., 1913, L, 1811; Gavini, Riforma Med., 1913, XXIX, 985, 1013, 1049, and 1075; McNeil, Jour. Am. Med. Assn., 1914, LXII, 529; Joltrain, Ann. de Méd., 1914, I, 337; Fagioli and Fischella, Berl. klin. Wchnschr., 1914, LI, 449; Kilgore, Jour. Am. Med. Assn., 1914, LXII, 1236; Much, Med. Klin., 1914, X, 811; Blechmann and Delort, Ann. de Méd. et Chir. Infant., 1914, XVIII, 406; Blechmann, Delort and Tulasne, Ibid., 465; Cannata, Pediatria, 1914, XXII, 481; Pusey and Stillians, Jour. Cutan. Dis., 1914, XXXII, 560; Noguchi, New York Med. Jour., 1914, C, 349; Muscel, Dersca and Friedmann, Münch. med. Wchnschr., 1914, LXI, 1272; Clausz, Ibid., 1933; Curti, Rif. med., 1914, XXX, 1264; Boas and Stürup, Hospitalstid., 1914, LVII, 1417; Trossarello, Gazz. d. osp., 1914, XXXV, 457; Wolff and Zeeman, Ibid., 811; Schippers, Ibid., 817; Kafka, Berl. klin. Wchnschr., 1915, LII, 15; Ross, Jour. Ment. Sc., 1915, LXI, 244; Sherrick, Jour. Am. Med. Assn., 1915, LXV, 404; Hanes, Am. Jour. Med. Sc., 1915, CL, 703; Kolmer and Broadwell, Jour. Immunol., 1916, I, 429; Fletcher, Lancet, 1916, II, 710; Fulton and Cummings, Am. Jour. Syph., 1917, I, 663; Cole and Paryzek, Jour. A. M. A., 1917, LXVIII, 1089.

² As this luetin reaction is an allergic one, it is not surprising to find that, in the primary and secondary stages, there are not sufficient antibodies present to produce the reaction. Although emulsion of ascitic agar itself may produce an intradermic reaction similar to that of luetin, yet this does not militate against the test as it shows a hypersensitiveness, which is itself rather characteristic of syphilis. See Stokes, Jour. Infect. Dis., 1916, XVIII, 415; Jour. A. M. A., 1917, LXVIII, 1092.

for many years, and in the course of which miscarriages have occurred, this reaction has failed to appear. Despite the absence of symptoms, mothers who have young syphilitic children have usually given the reaction. It remains to be determined in how far this reaction can be used to supplement the Wassermann reaction. It appears probable that the Wassermann reaction is more constant in the primary and secondary, and the cutaneous reaction in the tertiary and latent forms of syphilis.¹ It further appears that the Wassermann reaction is more directly and immediately affected by anti-syphilitic treatment than is the cutaneous reaction.²

Schick Reaction.

By means of this reaction individual susceptibility and immunity to diphtheria may be readily recognized in a great majority of cases. It consists in the intracutaneous injection of diphtheria toxin of definite dosage and the observance of the irritant action at the point of injection.

Technic.

In the first place the minimum fatal dose of the diphtheria toxin is determined for a guinea-pig of 250 grams weight.³ This amount is then so diluted with sterile physiologic salt solution that each c.c. of the dilution contains 0.2 of the minimum fatal dose. Of this dilution 0.1 c.c. (representing one-fiftieth of the minimum fatal dose) is injected into the skin by means of an accurately graduated syringe (1 c.c. record tuberculin syringe) with steel or platinum-iridium needle, which should be very fine, sharp and short-pointed. The site of injection is the skin of the outer side of the upper portion of the arm.

¹ See Robinson, *Jour. Cutan. Dis.*, 1912, XXX, 410. Sherrick (*Jour. Am. Med. Assn.*, 1915, LXV, 404) calls attention to the fact that recent or simultaneous administration of potassium iodid will cause a positive luetin reaction in non-syphilitic individuals. See Kolmer, Matsunami, and Broadwell, *Jour. A. M. A.*, 1916, LXXVII, 718.

² Irons has recently perfected a local skin reaction in cases of generalized gonorrheal infections, using as his vaccine a glycerin extract of autolyzed gonococci. After a few hours a papule is formed with a surrounding area of hyperemia, the maximum reaction being reached in 24 hours. Irons classifies as positive the reactions showing a total diameter of 5 mm. or over; negative when 3 mm. or less (*Jour. Infect. Dis.*, 1912, XI, 77). See, also, Fronstein, *Med. Obozr.*, 1913, LXXIX, 225. See Pryer and Sewell (*Jour. Lab. and Clin. Med.*, 1918, III, 525) for a cutaneous reaction for scarlet fever. See, also, Steinkopf, *Ztschr. f. Kinderhke.*, 1921, XXXI, 132.

Gay and Force (*Arch. Int. Med.*, 1914, XIII, 471) have introduced a skin reaction for typhoid fever, which consists in using a "typhoidin" solution, prepared by concentrating a glycerin bouillon culture of typhoid bacilli, and which is applied to the abraded skin. A difference of 2.5 mm. between the areolæ of the "typhoidin" point and the control point is taken as evidence of a positive reaction. See, also, Gay, *Am. Jour. Med. Sc.*, 1915, CXIX, 157. Pulay, *Wien. klin. Wchnschr.*, 1915, XXVIII, 1180; Kolmer and Berge, *Jour. Immunol.*, 1916, I, 409; Austrian and Bloomfield, *Arch., Int. Med.*, 1916, XVII, 663; Kilgore, *Ibid.*, 25; 1917, XIX, 263 and 276; Force and Stevens, *Ibid.*, 440; Gay and Lamb, *Jour. Lab. and Clin. Med.*, 1917, II, 217; Meyer and Christiansen, *Jour. Infect. Dis.*, 1917, XX, 357, 391 and 424. See Bigelow (*Arch. Int. Med.*, 1922, XXIX, 221) for a study of intracutaneous reactions in lobar pneumonia. Wildbolz (*Cor.-Bl. f. Schwiz. Aerzte*, 1919, XLIX, 793) has introduced an auto-urine intracutaneous test which seems to have some value in distinguishing an active from latent tuberculosis.

³ This toxin may be obtained from the larger laboratories handling biological products or may be procured from the health officers in the larger cities. In this latter case a definite amount of a known potency toxin is supplied together with a diluent fluid. After the toxin is diluted it deteriorates rapidly, so that it should not be used after 12 hours' standing. The undiluted toxin, obtained from the supply houses should be kept cool to prevent deterioration. Usually the potency of the toxin is stated on the package, so that the necessary dilution may be directly figured.

Reaction.

If the injection be properly done, one observes a white blister-like punctate-appearing wheal, which shows distinct markings of the openings of the hair follicles. This elevation (due to the traumatism) persists for a short time, all signs of it usually disappearing within an hour. If there is sufficient antitoxin in the blood of the subject to neutralize the small amount of toxin, no further changes should be observed. This is a negative reaction and indicates that the person is immune to diphtheria.

A positive reaction is noted in the gradually increasing redness and infiltration of the skin, a distinct circumscribed area of redness and infiltration measuring about 1 to 2.5 cm. being seen within 24 or 48 hours. This redness persists for several days and gradually fades, leaving a brownish pigmentation and slight scaling.

In some cases, especially in older children and adults, a *pseudo-reaction* is observed, which is not due to the action of the toxin but is, rather, an anaphylactic response to the protein substance of the diphtheria bacillus or, as Kolmer and Moshage believe, to trauma of a skin which is unduly sensitive. Some local redness and infiltration may be noted, but this comes on more rapidly than does the true reaction, the redness is less sharply outlined, the local infiltration is more marked and the signs disappear in 2 to 4 days with little or no pigmentation and no scaling. These false reactions are confusing, but should be easily differentiated by careful observation. To control the reaction, one may inject into the other arm 0.1 c.c. of bouillon diluted 1 to 10 or 1 to 1,000. An extreme sensibility of the skin will be indicated by slight local reaction in the control arm.

Significance.

In normal subjects a negative result indicates, in practically 100 per cent. of cases, the presence in the blood of diphtheria antitoxin and, hence, an immunity to diphtheria. A typical positive reaction points to an absence of antitoxin and a resulting susceptibility to infection.

From the reports of the literature, it is evident that about 80 per cent. of new-born children, 50 to 60 per cent. of children, especially those between the ages of 1 and 5, and 90 per cent. of adults contain sufficient antitoxin, as shown by this test, to make them immune to diphtheria. In the presence of diphtheria, the question of exposure of other children is an important one. It is evident that immunization by injection of antitoxin is indicated only in those giving positive Schick reactions. Further, by means of this test definite separation of the diphtheria cases from non-diphtheritic cases may be made, as patients with true diphtheria have little antitoxin in the blood and hence show a positive reaction. Likewise diphtheria carriers may be separated from actual diphtheritics, as the former usually have a large amount of antitoxin in the blood and, therefore, give negative reactions.

There can be little question but that this test will prove invaluable in hospitals in selecting internes and nurses to look after the diphtheria patients. Moreover, the question of isolation of actual diphtheria patients becomes a much easier matter, with the knowledge that those showing a negative Schick reaction are immune to diphtheria and need not, therefore, be removed from

chance of infection. The economic as well as the diagnostic value of this test seems to be very great.¹

VIII. SERO-DIAGNOSIS

In the attempt to enlarge our diagnostic procedures advantage has been taken of many of the principles previously discussed. The methods evolved are among those most frequently employed and are reliable within certain small limits which will be mentioned later. They are all based upon certain peculiar properties possessed by the serum of patients with infectious diseases and are, therefore, of special importance in differential diagnosis.

A. Agglutination Reactions.

Since the work of Gruber and Durham and of Pfeiffer and Kolle in 1896, the fact has been well established that the serum of an animal, which has been rendered immune to certain bacteria, shows the property of agglutinating or

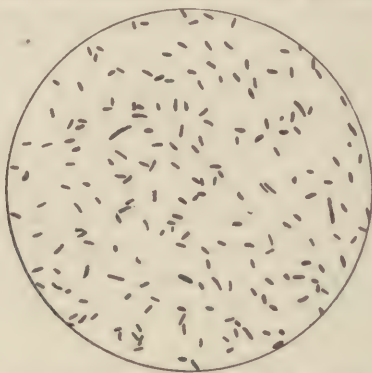


FIG. 156.—*Bacillus typhosus* at beginning of Widal test. (Da Costa.)

clumping homologous bacteria, owing to the production of immune bodies, the so-called agglutinins. This reaction takes place in high dilutions of the

¹ Schick, Münch. med. Wchnschr., 1913, LX, 2608; Groër and Kassowitz, Ztschr. f. Immunitätsf. u. exper. Therap., 1914, XXIII, 108; Kassowitz and Schick, Ztschr. f. d. ges. exper. Med., 1914, II, 305; Otto, Deutsch. med. Wchnschr., 1914, XL, 542; Schick, Kassowitz and Busacchi Ztschr. f. d. ges. exper. Med., 1914, IV, 83; Park, Zingher and Serota, Arch. Pediat., 1914, XXXI, 481; Jour. Am. Med. Assn., 1914, LXIII, 859; Veeder, Am. Jour. Dis. Child., 1914, VIII, 154; Kolmer and Moshage, Ibid., 1915, IX, 189; Weaver and Maher, Jour. Infect. Dis., 1915, XVI, 342; Bundesen, Jour. Am. Med. Assn., 1915, LXIV, 1203; Graef and Ginsberg, Ibid., 1205; Moody, Ibid., 1206; Kolmer and Moshage, Ibid., 1915, LXV, 144; Zingher, Ibid., 329; Moffett and Conrad, Ibid., 1010; Birnberg, St. Paul Med. Jour., 1915, XVII, 204; Levinson and Blatt, Arch. Diag., 1915, VIII, 201; Zuckermann, New York Med. Jour., 1915, CII, 808; Weaver and Maher, Jour. Infect. Dis., 1915, XVI, 342; Park and Zingher, Jour. A. M. A., 1915, LXV, 2216; Levinson, Ill. Med. Jour., 1915, XXVIII, 405; Sprenger, Ibid., 1916, XXIX, 447; Koplik and Unger, Jour. A. M. A., 1916, LXVI, 1195; Bessau and Schwenke, Monatsschr. f. Kinderhke., 1916, XIII, 393; Kolmer, Jour. Immunol., 1916, I, 443; Bullen, N. Y. State Jour. Med., 1916, XVI, 208; Griswold, Jour. Lab. and Clin. Med., 1916, I, 441; Weaver and Rappaport, Jour. A. M. A., 1916, LXVI, 1448; Zingher, Ibid., 1617; Am. Jour. Dis. Child., 1916, XI, 260; Ibid., 1917, XIII, 247; Arch. Int. Med., 1917, XX, 392; Renault, Bull. de l'Acad. de Méd., 1920, LXXXIII, 130; Laverge and Zoeller, Bull. de la Soc. Méd. des Hôp., 1920, XLIV, 954; Blum, Am. Jour. Dis. Child., 1920, XX, 22; Zingher, Jour. Lab. & Clin. Med., 1920, VI, 117; Armand-Delille and Marie, Presse Med., 1921, XXIX, 43; White, Boston Med. & Surg. Jour., 1921, CLXXXIV, 246; Renault, Bull. de la Soc. Méd. des Hôp., 1921, XLV, 529; Ekvall, Upsala Läk. Förländ., 1921, XXVI, 219; Hannah, Public Health Jour., 1921, XII, 250; Park, Am. Jour. Dis. Child., 1921, XXII, 1; Comby, Arch. de Méd. des Enfants, 1921, XXIV, 624; Dickinson, Lancet, 1922, I, 312; Zingher, Jour. Am. Med. Assoc., 1922, LXXVIII, 400; Meyer, Ibid., 716; Kellogg, Jour. Am. Med. Assoc., 1922, LXXVIII, 1782.

serum and is practically specific for any given organism. Widal,¹ in the same year, applied this principle to the diagnosis of typhoid fever, showing that clumping and loss of motility of the typhoid bacilli occur when a suspension of the actively motile types is treated with an homologous immune serum at such a dilution that normal or non-homologous serum does not react.

1. Gruber-Widal Test.

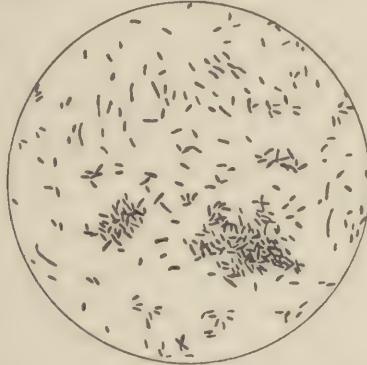


FIG. 157.—A pseudo-Widal reaction. (*Da Costa.*)

Cultures.

The cultures of the typhoid bacillus must be fresh and must show many actively motile organisms in the hanging-drop specimens, if they are to be used in the microscopic method. It is advisable always to use cultures containing about the same number of bacilli, as the reaction is somewhat quantitative. Thus, if few bacilli be present, they may be clumped by a small

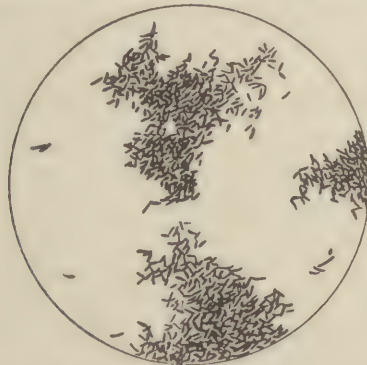


FIG. 158.—A positive Widal reaction. (*Da Costa.*)

amount of agglutinin both specific and non-specific, while if the number be very large, the specific agglutinin may not be sufficient to cause marked clumping and loss of motility of the organisms. Great differences toward agglutination exist in different strains of the typhoid bacillus, so that it is essential always to employ, for diagnostic purposes, strains which have passed through several generations on artificial media.

A stock culture of the bacillus is kept in sealed tubes of nutrient agar in a

¹ Bull. méd. Paris, 1896, X, 618 and 766.

cool dark place and, from this stock, fresh agar cultures are made every few weeks as occasion may require. From these stock agar cultures, bouillon tubes are inoculated and incubated for 15 to 24 hours. These latter are used for the agglutination tests. These bouillon cultures should be equally cloudy throughout and show no pellicle formation or gross clots of bacilli. It is wise to make up fresh bouillon cultures every other day so as to be certain of the maximum motility of the organisms.¹

In the use of the macroscopic method cultures killed by heat, formalin, carbolic acid, thymol, etc., may be employed. Many laboratories throughout the country supply these cultures for general work. Ficker's "Typhus diagnostikum" is one of the widely used types. For self-evident reasons it is wise to have some standard strength of these cultures. Hastings advocates a mixture of 5 c.c. of 5 per cent. carbolic acid solution, 10 c.c. of glycerin, and 85 c.c. of physiologic salt solution to which are added the organisms scraped from the surface of two 24-hour agar-slant cultures of *bacillus typhosus* (the bacilli being rubbed into the mixture with a spatula). Bass advises a suspension of 10,000 million dead typhoid bacilli per c.c. in 1.7 per cent. NaCl solution to which 1 per cent. formalin is added.

Obtaining the Blood.

In this procedure much depends upon the custom of the worker as to the method followed. As this reaction is a pure serum one, it is much more logical to use the serum than the whole blood, if one has any choice in the matter.

The simplest and best clinical method in the use of a small capillary tube (made from $\frac{1}{4}$ -inch glass tubing) with a central bulbar enlargement into which the blood (15 to 20 drops) is drawn by capillarity from a puncture of the ear. The tube is then laid flat until coagulation occurs and the serum separates. To hasten this process, the end of the tube, which contains no blood, may be sealed in the flame and the tube centrifuged. If it is desired to send the specimen to the laboratory, seal both ends and pack in such a way as to insure it against breakage. In this connection it may be well to recall that Lyons² has shown the possibility of obtaining a culture of the *bacillus typhosus* from the clot settling in such tubes. This may be done by the method previously outlined. Another method of obtaining the serum is to apply a cantharides blister which will furnish enough serum in 6 to 12 hours for many tests. Venous puncture may, also, be employed, but this is rarely advisable as sufficient serum may be more easily obtained in the above ways.

Some workers use the whole blood instead of the serum. If this be done, the leucocytometer may be employed, using physiologic salt (0.9 per cent.) solution as the diluent. Draw the blood from a puncture in the ear to the mark 1 and the diluent to 10. This gives a dilution of the whole blood of 1 to 10 or 1 to 20 of the plasma, as the plasma forms approximately 50 per cent.

¹ Riemer (Münch. med. Wchnschr., 1913, LX, 908) calls attention to the fact that typhoid bacilli show diminished agglutination if grown in alkaline media. See, also, Gay and Claypole, Arch. Int. Med., 1913, XII, 621; Jour. Am. Med. Assn., 1913, LX, 1141; Bull and Pritchett, Jour. Exper. Med., 1916, XXIV, 35; Ishii, Jour. Bact. 1922, VII, 9.

² Arch. Int. Med., 1909, IV, 64.

of the whole blood. This dilution may then be used directly for the later work by mixing with an equal volume of the typhoid suspension.

More frequent, possibly, in the hands of workers in municipal or the larger clinical laboratories, is the employment of dried blood sent to them on glass slides, bits of paper, mica, etc. Such specimens may be easily transported but can not yield anything but an approximate result, as an accurate dilution is out of the question unless very careful weighing of the blood is carried out. A large drop of the dried blood is dissolved in 10 drops of water, the dilution being called 1 to 10. If the blood be upon paper, the dilution must be made largely by guess, experience teaching an approximate dilution from the color of the mixture. These dilutions will, of course, be doubled when the test is applied.

Dilution of the Serum.

The methods of diluting the whole fresh blood and the dried blood have already been given. As diluting pipets for the serum, one uses small ($\frac{1}{4}$ inch) glass tubing which has been drawn into a long capillary at one end. A rubber bulb fits over the larger end and provides the suction. It is good practice always to have a number of these pipets on hand so that they may be discarded or thoroughly cleaned before a second use. If the same pipet be employed for different cases, a contamination may arise to such an extent that erroneous results obtain.

A few drops of the serum, collected as above, are drawn into the capillary tube by suction. From this tube the serum is then dropped into the mixing vessels, preferably watch crystals. Two dilutions are usually made in the writer's laboratory, namely 1 to 25 and 1 to 50. Allow 1 drop of serum to fall into each of two watch crystals, the remaining serum in the capillary being blown into the original collecting tube. Now add, from the same pipet used in dropping the serum in order to insure drops of the same size, to one of these drops of serum 24 drops of physiologic salt solution and to the other 49 drops of the same diluent. Mixtures of these diluted sera with an equal volume of typhoid suspension, as employed in the test, give final dilutions of 1 to 50 and 1 to 100.

Microscopic Reaction.

The serum (preferably), whole blood, or dried blood is diluted as previously described. Mixtures are then made of these dilutions with an equal volume of typhoid suspension. By means of a platinum loop of stiff wire, a loopful of the diluted serum (1 to 25) is placed on the center of a cover-slip. After burning the serum from the loop, a loopful of the typhoid suspension is transferred to the same cover-glass and the two fluids thoroughly mixed by means of the loop. The dilution of the serum will then, evidently, be double its original dilution, or 1 to 50. The ordinary hanging-drop preparation is then made by inverting the cover-slip over the hollow of the slide, which has been ringed with vaseline. Instead of using the hanging-drop method, some prefer to place the cover directly upon the slide. Usually the specimen does not dry sufficiently to introduce errors, but the results are certainly not as characteristic by this procedure in all cases.

The specimen is best studied with the high-power lens, using a strong light. The organisms will be seen, in specimens properly lighted, as free actively motile bacilli moving with their rapid darting motion through the field. In the course of 45 to 60 minutes, or often in much less time, the organisms will be seen to be collected in clumps and to be motionless, except for a slight vibratory movement. This is a typical positive reaction, namely marked clumping with complete loss of motility in one hour at a dilution of 1 to 50. Such a reaction is practically never due to any other disease than typhoid fever, if the culture of the bacillus be a pure one.

In some cases marked agglutination with incomplete loss of motility obtain. In the writer's opinion this can be interpreted only as a doubtful or incomplete reaction. The probability is that typhoid fever is present, but one must admit the possibility of group agglutinins, due to the paratyphoid bacillus, as well as agglutinins of other infections. When dried blood is used, a slight tendency to agglutination may be observed, due to the presence of small particles of fibrin, so that in such cases the loss of motility should be demanded even more rigorously than when serum is used, before a positive result is reported. It has been shown that the serum of many healthy patients or that of subjects suffering with diseases other than typhoid fever frequently show a reaction similar to that described above. This statement is true only when the question of dilution of the serum is taken into account. The characteristic Widal reaction is shown at a dilution of 1 to 50 or higher, while in other diseases, with the exception of paratyphoid, the reaction practically disappears (the typhoid bacillus being, of course, used) at a dilution of 1 to 20. The element of time is also of great importance. In typhoid fever, the reaction may occur instantly at 1 to 10 or 20, and in one-half to one hour at 1 to 50, yet a period ranging from one-half to one hour is necessary with other sera at a dilution of 1 to 10 or 20. On the other hand, there are cases of undoubted typhoid fever that show no agglutination at 1 to 10 or 20, while at 1 to 50 this is marked. What occurs here is uncertain, some crediting the paradox to bacteriolysis of the organisms at the former dilutions.

Macroscopic Reaction.

The method is based on the fact that the agglutination of typhoid bacilli by a potent homologous serum is visible to the naked eye, the clumps of bacilli settling to the bottom of the test-tube leaving a clear supernatant fluid.

If living cultures of bacillus typhosus are employed, the best technic is the following: Make up the serum dilutions as previously described, using 1 to 50 and 1 to 100 dilutions. To these diluted sera, contained in small sterile test-tubes (11 cm. long by 88 mm. wide), is added a loopful of typhoid bacilli scraped from the surface of a 24-hour agar culture. The loop is rubbed against the inner surface of the test-tube until the bacteria have been suspended in the serum. The turbid suspension is then thoroughly shaken until it becomes uniformly cloudy, no clumps of bacteria being seen, and is placed in the incubator at 37°C. for one hour. The tubes are then removed and examined by strong transmitted light. The agglutinated bacteria may easily be recognized by their granular flocculent appearance. General clumping

should obtain in one hour at a dilution of 1 to 50, while complete precipitation (settling) should follow in 24 hours, a clear supernatant fluid being left behind. This is the positive reaction. A negative result is shown by no clumping and little, if any, loss in the turbidity of the mixture.

For the general practitioner, who desires to use the macroscopic method, the employment of cultures killed by heat or chemical means is recommended, as it obviates the necessity of an incubator, culture media, or microscope and permits him to have on hand material for his tests. Any of the suspensions of killed organisms may be used, Ficker's and Hastings' being widely employed. Dilute the serum as previously described, the dilutions in this case being 1 to 25 and 1 to 50. To this diluted serum ($\frac{1}{2}$ c.c.), contained in the small test-tubes, add an equal volume of the suspension of killed bacteria and examine as above mentioned. The clumping should be observed in one hour and complete clearing should obtain in 24 hours, if the reaction be positive. This method gives very reliable results and is very simple.

Method of Bass and Watkins.

These workers¹ have introduced a modification of this macroscopic agglutination test which is simple, reliable, and quick. It has the advantage that it can be carried out at the bedside, the result being known in 2 to 3 minutes. Little equipment is required. The suspension consists of 10,000 million killed typhoid organisms per c.c. in 1.7 per cent. NaCl solution to which 1 per cent. formalin is added.

Allow a full drop of blood to fall into 4 drops of water and mix thoroughly. Instead of this, one may make a blood-smear (using approximately $\frac{1}{4}$ drop of blood) and dissolve this on the slide with 1 drop of water, the mixture being stirred with a tooth-pick or similar substance. With this diluted blood (1 to 4) mix an equal amount of the above suspension of typhoid bacilli on a glass slide. Tilt the slide from side to side so as to keep the mixture flowing back and forth. If the reaction is positive, a grayish mealy sediment appears within one minute, usually in less time. This sediment appears in the fluid around the edges and tends to collect there. If the agitation is continued, the clumps increase in size for 2 to 3 minutes. If the reaction does not appear in this time, it will not show up at all. When the reaction is negative, no agglutination occurs and the mixture remains as clear and unchanged as when placed on the slide.

Recently Michaelis² has advocated a new agglutination reaction for the bacillus typhosus as well as other bacteria. This is based on the fact that bacteria are agglutinated by acids, the rate and degree being dependent upon the concentration of hydrogen ions present. This test promises much in the future as it depends entirely upon chemical factors which are more easily and more exactly controlled than are the uncertain biologic units with which we usually work.³

¹ Arch. Int. Med., 1910, VI, 717. Gates Jour. Exp. Med. (1921, XXXV, 63) employs the centrifuge in studying agglutination reactions. See, also, Gilbert and Moore, Jour. Lab. and Clin. Med., 1922, VII, 547.

² Folia Serologica, 1911, VII, 1010; Deutsch. med. Wchnschr., 1915, XLI, 241.

³ See Beniasch, Zeitsch. f. Immun.-Forsch., 1912, XII, 268; Gillespie, Jour. Exper. Med., 1914, XIX, 28

Time of Appearance and Disappearance of the Reaction.

The time at which a Widal reaction may appear in the course of a typhoid is of some importance. The earliest date of appearance of a positive reaction is rather hard to determine, as workers differ in their interpretation of the beginning of the disease. Definite reports of positive results as early as the second day have been given by Fraenkel and others. Although this reaction may appear before the rose spots, splenic tumor, or diazo reaction, yet it is practically never found when the blood shows a normal or an increased number of leucocytes, a leucopenia with a relative lymphocytosis being associated with a positive Widal in practically all cases. A differential blood count may, therefore, be of great value when the means of making an agglutination test are not at hand. Wood was able to obtain positive results in only 10 per cent. of his cases in the first week of the disease. The majority of observers are agreed that the first appearance of this reaction is in the second week of the disease in most cases. In a certain number, it may not appear till the third or fourth week the highest agglutinations frequently being seen toward the end of the disease and in the early stages of convalescence. It is very rare not to obtain a positive reaction at some stage of the disease, providing the tests are made at different intervals. It is possible that cases reported, in which no Widal reaction was observed throughout the course of the disease, may be due to faulty technic or to insufficient tests. A single negative test, of course, proves nothing.

The persistence of the Widal reaction is likewise variable. Little seems to depend upon the severity of the attack as to the time of disappearance of the reaction. Usually this reaction persists for 1 to 2 months, although it may obtain for 4 to 6 months or even for years. It is undetermined whether these latter cases are due to reinfection rather than a continuation of the agglutination powers of the serum. Reports are extant of cholelithiasis in which typhoid bacilli were isolated from the gall-bladder as long as 18 years after an attack of typhoid, the Widal reaction being positive in these cases. In this connection it is to be said that "typhoid-carriers" do not show a positive Widal as a rule.

Specificity of the Reaction.

From the standpoint of the biologic theory, the typhoid bacillus is agglutinated only by an homologous immune serum. Practically, we find that this is the case within certain limits, if all conditions are properly met. A positive Widal reaction is not infrequently met with in cases of jaundice, in which no specific disease is clinically evident.¹ However, it is certain that most, if not all, of these cases of jaundice are actually secondary to a typhoid cholecystitis with its accompanying sequelæ. Such patients may have an "ambulatory" typhoid or may have no clinical signs other than a positive Widal.

¹ See Mazza, Gazz. d. osp., 1914, XXXV, 921; Perlmann, Münch. med. Wchnschr., 1915, LXII, 435; Stuber, Ibid., 1173; Bernard and Paraf. Presse méd., 1915, XXIII, 333; Blassberg, Wien. klin. Wchnschr., 1915, XXVIII, 1314; Davison, Jour. A. M. A., 1916, LXVI, 1297; Pollak, Wien. klin. Wchnschr., 1916, XXIX, 1204; Baerthlein, Münch. med. Wchnschr., 1916, LXIII, 1564; O'Farrell, Lancet, 1916, II, 970; Levy and Vallery-Radot, Ann. de Méd., 1916, III, 504 and 515.

Further, in many cases of infection with bacteria of the colon group, a "collateral agglutination" may obtain, that is agglutination of several members of the group may occur with one serum. Cases are undoubtedly met with in which the infection is due to the paratyphoid bacillus and in which the agglutination of the typhoid bacillus is stronger than the paratyphoid; while, on the other hand, cases of true typhoid may show a stronger reaction with bacillus paratyphosus than with the bacillus typhosus. According to Lentz, the time of such associated agglutinations is important in this connection. The paratyphoid bacillus is agglutinated more quickly by a paratyphoid serum than is the typhoid bacillus by the typhoid serum.

A positive reaction, all conditions outlined above obtaining, indicates infection with the bacillus typhosus in practically all cases. This test can not be regarded as of great value in the early stages, as only a very small percentage of cases show early agglutination reactions. It is especially serviceable in the differential diagnosis from the second week of the disease on to convalescence. In the ambulatory types or in localized typhoid infections, especially in the gall-bladder, this test is of great value. A negative reaction should not be interpreted as excluding typhoid, as many typical and severe cases show no reaction until late in the disease.¹ Like all laboratory tests, it must be used only in connection with the clinical findings, if the best results are to follow. The attending physician is the one to interpret the result, not the laboratory worker.

2. Diseases Other than Typhoid Fever.

Other organisms, beside the bacillus typhosus, show such agglutination reactions. The technic is the same and the results comparable, the same specificity being shown. It is especially in the realm of infections with members of the colon group that these tests are, frequently, uncertain, yet, here also, careful variation in the dilution of the serum and observation of the time and degree of agglutination will lead to a positive result, which will be of the greatest clinical value.

The organisms, frequently identified by these reactions, are the paratyphoid bacilli, cholera spirillum, Shiga bacillus, bacillus of plague, Malta-fever, tuberculosis, pertussis, influenza, and the diplococcus of pneumonia.²

¹ The widespread use of typhoid vaccine as a preventive inoculation has introduced a large possible error into this reaction. The serum of injected, (immune) patients shows a positive Widal reaction very quickly. This condition persists for a varying period, so that blood cultures or Austrian's ophthalmo-reaction (see p. 706) should be used in such cases. See Moon, Jour. Am. Med. Assn., 1913, LX, 1764; also Kellermann, Münch. med. Wchnschr., 1914, LXI, 2433; Hirschbruch, Deutsch., Med. Wchnschr., 1915, XLI, 525; Hamilton, Jour. A. M. A., 1915, LXV, 1873; Tonnel, Lyon Méd., 1916, CXXV, 105; Chantemesse, Bull. de l'Acad. Méd., 1916, LXXVI, 140; Gautier and Weissenbach, Presse Méd., 1916, XXIV, 413; Walker, Lancet, 1916, II, 896; Rist, Ann. de Méd., 1916, III, 54; Salomon Presse Méd., 1916, XXIV, 91; Conradi and Bieling, Deutsch. Med. Wchnschr., 1916, XLII, 1280; Danila and Stroe, C. R. soc. biol. Paris, 1916, LXXIX, 108; Meyer and Kilgore, Arch. Int. Med., 1917, XIX, 293; Rist, Jour. Lab. and Clin. Med., 1917, III, 1; Klemperer and Rosenthal, Ztschr. f. klin. Med., 1918, LXXXVI, 1; Krumbhaar and Smith, Jour. Infect. Dis., 1918, XXIII, 126.

² Kolmer (Jour. Exper. Med., 1913, XVIII, 18) has shown that there is no appreciable amount of agglutinin for spirillum (treponema) pallidum in the sera of secondary and tertiary syphilis. See, however, Zinsser and Hopkins (Jour. Exper. Med., 1915, XXI, 576, and Kissmeyer, Deutsch. med. Wchnschr., 1915, XLI, 306.

B. Precipitin Reaction.

This reaction is based upon another fact well established in serum pathology. It has been proven that the serum of an animal, injected with blood or blood-serum of another animal, shows the property, when added to an homologous serum, of precipitating the albumin of this serum in the form of a light flocculent precipitate. This same peculiarity has been observed after injection of exudates or transudates or of pure proteins both animal and vegetable.¹ These reactions are, therefore, specific for the type of protein injected, within certain definite limits. Although this test has been used in the detection of certain albuminous substances excreted in the urine (see p. 290), yet its chief interest to the laboratory worker is its application to the medico-legal detection of blood and blood-stains.²

The work of Wassermann and Uhlenhuth has shown that blood-stains may reveal their origin from any particular species of animal by application of this test. It is evident that one must, of course, prove that such stains are really due to blood by other methods, as this biologic test is merely for specific proteins and not for blood as such. These latter tests will be given in a later section. In this discussion the writer quotes largely from Uhlenhuth and Weidanz.³

The Anti-serum.

This is the serum of the animal immunized against the proteins of the blood, exudates or secretions of a particular species of animal. These proteins are usually those of the human as this type is of especial importance in medico-legal investigations. However, in the larger laboratories where many different serological tests are being frequently made, animals are kept on hand, which have been immunized against the proteins of most domestic animals, so that an absolute differentiation and identification of a stain may be made.

The preparation of the anti-serum is as follows: A rabbit is injected intraperitoneally with 5 to 10 c.c. of whole blood or serum. At intervals of 3 to 5 days other similar injections are given until six or eight in all have been made. About a week after the last injection, a few drops of blood are drawn from a puncture in the ear of the animal and are allowed to clot in a small test-tube. The potency of the serum is then tested by permitting a few drops of the separated serum to fall into a small test-tube containing about 1 c.c. of a 1 to 1,000 dilution of dried homologous blood in physiological salt solution. In this preliminary potency test as in the later precipitin test it is important that the dilution of the solution under investigation should be about 1 to 1,000. One may recognize this proper dilution by the fact that the

¹ See last chapter for a discussion of the phenomena of anaphylaxis brought about by such injections.

² See Hektoen, *Jour. Infect. Dis.*, 1914, XIV, 403; Bing and Trier, *Ugesk. f. Laeger*, 1914, LXXVI, 2117; Bulger, *Jour. Infect. Dis.*, 1916, XIX, 832; Smith, *Jour. Med. Res.*, 1916, XXIV, 169; Bayne-Jones, *Jour. Exper. Med.*, 1917, XXV, 837; Stokes and Stoner, *Bost. Med. and Surg. Jour.*, 1917, CLXXVII, 65; Hektoen, *Jour. A. M. A.*, 1918, LXX, 1273; Kominami and Kusakari, *Kyoto Igaku Zasshi*, 1918, XV, 4; *Chem. Abs.*, 1919, XIII, 2920; Hektoen, Fantus and Portis, *Jour. Infect. Dis.*, 1919, XXIV, 482, have applied this test to detection of occult blood in feces.

³ *Prakt. Anleitung z. Ausführung des biol. Eiweissdifferenzierungsverfahrens*, Jena, 1909. See, also, Leers, *Die forensische Bluntuntersuchung*, Berlin, 1910.

solution forms a layer of foam on shaking and, further, on heating and adding to 1 c.c. of the solution a drop of 25 per cent. nitric acid, only a slight opalescence obtains. If the addition of the anti-serum to the 1 to 1,000 dilution of dried blood causes a turbidity in 1 to 2 minutes, the serum is sufficiently active for the test and the animal may be bled.

The blood is now withdrawn, either in small amount as desired for immediate use or the animal may be completely bled. The blood is collected in wide-mouth test-tubes, which should be then plugged with cotton and placed in a slanting position until the blood is coagulated. The serum, which must be clear and sterile, is removed to small test-tubes, which are plugged with cotton, sealed with paraffin, and stored in a cool place. This is the anti-serum. It should be sufficiently potent to produce a turbidity almost at once, at latest in 1 to 2 minutes, in a 1 to 1,000 dilution of homologous blood in 0.9 per cent. NaCl solution.¹

Solution of the Blood or Stain.

As a preliminary precaution, it is to be emphasized that all vessels and instruments used should be thoroughly clean and sterile and that all fluids be absolutely clear.

If the blood or exudate be dried upon solid material, such as glass, knife blades, stone, wood, etc., it is carefully scratched off with a sterile instrument. This material is powdered and dissolved, as far as possible, in chemically pure physiologic salt solution contained in a sterile test-tube. If only traces of the stain are present, one may form a well of wax about the spots and place in this some of the salt solution. It is absolutely essential that no other solvent than 0.9 per cent. NaCl solution be used, as otherwise erroneous reactions may occur or the usual precipitin reaction may not obtain.

If the stain has penetrated clothing or other soft material, the spot is cut out with scissors, is finely divided, teased with a needle and placed in a small watch-glass or test-tube with a small amount of salt solution for one hour, or, if the stain be very old or has been exposed to marked changes in the weather, for 24 hours in a cool place.

The fluid obtained in either of the above ways is filtered, first through hardened filter-paper (Schleicher and Schüll, Nr. 575, 603, or 605) and then through a small Berkefeld filter. This filtrate, which must be perfectly clear, should be neutralized to litmus either by the addition of sodium carbonate or tartaric acid solutions, as the case may be. Further, this filtrate should show distinct foam-formation on shaking. This is not always the case if the stain has been exposed to the sun and dust or has dried either upon fatty material or iron. Under these conditions, the precipitin reaction may not be seen in the usual time, but will obtain, as a rule, in five minutes.

As previously stated, the dilution of this physiologic salt extract of the stain should be approximately 1 to 1,000. If sufficient stain can be scratched from the surface of the material upon which it is found, this may be weighed

¹ It is important to remember that such serum may contain a small amount of antigen (along with the precipitin) which may persist as long as 15 days after the last injection. See von Dungern, *Centralbl. f. Bakteriol. 1te Abt., Orig.*, 1903, XXXIV, 355; Uhlenhuth and Weidanz, *Loc. cit.*, 221; Zinsser and Young, *Jour. Exper., Med.*, 1913, XVII, 396.

and proper dilution made. Such procedures are usually impossible, so that we use the approximate method given on previous page.

Technic of the Test.

The reaction is carried out in the small test-tubes, which should be approximately of the same size and thickness, should be thoroughly clean and sterile, should be numbered and arranged in a series of 10 tubes in a small rack.

In tubes 1 and 2 are placed 1 c.c. of the above solution of the stain to be tested.

In tube 3, 1 c.c. of a 1 to 1,000 dilution of known fresh human blood in physiologic salt solution.

In tube 4, 1 c.c. of a 1 to 1,000 dilution of dried human blood in 0.9 per cent. salt solution.

In tube 5, 1 c.c. of sterile physiologic salt solution.

In tubes, 6, 7, 8, and 9, 1 c.c. each of a 1 to 1,000 dilution of fresh or dried blood of such domestic animals as may be easily obtained, as chicken, dog, horse and sheep blood.

In tube 10, 1 c.c. of a physiologic salt extract of the material upon which the stain was found.

To each of the above tubes, with exception of tube 2, is now added from a carefully graduated pipet 0.1 c.c. of the anti-serum, which has been tested for its potency as above described. To tube 2 is added 0.1 c.c. of normal clear rabbit serum. The anti-serum must be carefully added in such a way that it runs down the side of the tube and does not drop directly into the fluid. Or a fine pointed graduated tube may be introduced below the fluid in the test-tube and the anti-serum allowed to flow out. In this way the line of contact of the fluids is made somewhat sharper and the reaction is, perhaps, more easily observed. After this addition the tube must not be shaken. The reaction is carried out at room temperature.

If the reaction be positive, a distinct clouding or turbidity will be observed immediately, or at latest within two minutes, in tubes 1, 3, and 4 (all of which contain human blood), while the other tubes should remain perfectly clear. If the anti-serum has been properly added, the turbidity may be visible as a distinct contact ring which gradually increases upward. A negative reaction is shown by the lack of turbidity in any of the tubes.

Specificity of the Reaction.

Although this test is extremely delicate, yet it may happen that a stain, definitely identified as a blood-stain by other tests, may not react typically to the precipitin test, owing to the small amount of protein matter in the salt extract. This is especially the case if the protein has been rendered insoluble by heat or destroyed by chemical agents or by putrefaction. Even in such cases a positive reaction may occasionally be obtained, Uhlenhuth and Beumer reporting positive results after blood had been subjected to putrefaction for two years.

As far as the influence of time upon this reaction is concerned, it may be said that blood-stains over 50 years old have been detected. The whole point, in this connection, is whether any trace of soluble protein remains. If so, the

test will react positively, providing the dilution and the time of reaction be carefully regulated to suit the lessened concentration.

Heterologous precipitin reactions do not occur if the conditions of the test are properly maintained. If more concentrated blood solutions are used and the amount of anti-serum increased, such erroneous reactions may obtain. It is true that confusing reactions may arise in the differentiation of the blood of the closely related animals such, for instance, as the horse and donkey; goat, sheep and ox; dog and fox; hare and rabbit; chicken and pigeon; and especially man and the anthropoid apes (orang-outang, gorilla and chimpanzee). However, the point of great medico-legal importance is that such close differentiations are rarely called for, as the question at issue is, usually, whether a given stain is or is not due to human blood. As the apes are relatively scarce in this country, confusion is seldom probable. This reaction may, therefore, be regarded as practically specific for the especial type of blood protein with which the immune serum reacts (all details of the test being properly regulated), only the proteins of the very closely related animals responding to the test in a way which might leave the slightest room for doubt as to the origin of a stain or of a protein.

The substance on which the stain is found may, often, exert some retarding action on the test. Stains upon cloth or paper usually show prompt results, although those upon wall-paper may, at times, react atypically owing to the influence of the chemical of the dyes. Strong alkalis or acids prevent the reaction, but these are thrown out by neutralization before the test is applied. Tannic acid, derived from stains upon leather may prevent the reaction, although this is not always the case. Iron rust may retard but does not prevent the reaction. If the stain be upon soft clay, mortar, lime or fresh plaster so that an intimate mixture has occurred, the reaction will probably not obtain, according to Graham-Smith and Sanger, but if the stain be upon hard well-dried mortar no influence is seen, as demonstrated by Biondi.

As previously stated, this active immune serum reacts promptly with the protein elements of exudates, sputum, pus, urine, feces, nasal and bronchial secretions, vaginal, lochial and seminal fluids, etc., so that a positive precipitin reaction means merely that a protein is present, which has the same origin as that against which the animal furnishing the anti-serum was immunized.¹ Whether or not this is a blood protein is, in no way, a part of the test. This must be determined by other tests.

C. Complement Fixation Test.

As previously stated, the serum of an animal, which has been injected with bacteria or with washed blood corpuscles of an animal of a different species, acquires the property of dissolving homologous cells through the development of antibodies or immune bodies, which are specific for the cells injected. This process is known as bacteriolysis or hemolysis. Such sera are known as lytic; bacteriolytic, if the action is directed toward bacteria, hemolytic, if

¹ Robertson and his pupils have contributed some interesting articles on the blood relationship of animals as displayed in the composition of the serum proteins. See Robertson, *Jour. Biol. Chem.*, 1912, XIII, 325; Woolsey, *Ibid.*, 1913, XIV, 433; Thompson, *Ibid.*, 1915, XX, 1; Briggs, *Ibid.*, 7; Sutherland (*Indian Jour. Med. Res.*, 1915, III, 205) reports a study of 6566 articles examined for blood stains.

toward blood cells. It is to be remembered, in this connection, that fresh blood serum of many species is hemolytic for the red cells of some, but not all, other species, the degree being relatively small. Moreover, we find that the blood of the same species may be markedly hemolytic for the homologous cells, as evidenced by the effects occasionally observed after transfusion. It is, therefore, evident that we may have in any serum both the immune and the natural hemolytic agents, the former being much the more effective.

It has been shown that hemolysis, by means of an immune serum, may be prevented by heating the serum to 55 to 56°C. for one-half hour, the process being known as inactivation. Addition of a small amount of a fresh, isologous serum will restore (reactivate) the hemolytic activity. Hence, two separate factors must be concerned in hemolysis, the one a thermostable (heat-resisting) substance, the other a thermolabile (destroyed by heat) product. The first of these is called the amboceptor, the latter, the complement. Amboceptor is usually still active in serum which has been kept for a long period, while complement disappears from the serum in a short time even though it be kept on ice. The function of the amboceptor is to sensitize the cells, while that of the complement is to dissolve the cells so sensitized. Hemolysis is impossible without the combined action of both factors, either one singly having no effect. Complement can combine with the cell only through the medium of amboceptor, while this union does not take place unless amboceptor and antigen (the blood cells) are homologous. In other words, the cells can be hemolyzed only by homologous serum in the presence of complement. It is evident, on the other hand, that hemolysis must occur when the proper hemolytic trinity is present. The amboceptor and complement bear definite quantitative relations to one another in this process, an increase of one factor permitting the use of a less amount of the other.

Complement may combine itself equally well with bacteriolytic or hemolytic amboceptor providing the homologous antigen be present. If, therefore, we mix bacterial emulsions with the corresponding inactivated immune serum and complement, this latter must attach itself (become fixed) to the bacterial cell through the medium of the amboceptor, so that the later addition of hemolytic amboceptor and washed cells will not result in hemolysis, owing to the fact that the complement originally present has been fixed to such an extent that none is left to combine with the hemolytic amboceptor. This phenomenon, discovered by Bordet and Gengou, is known as "fixation of complement" or as "deviation of complement." It is decidedly a quantitative as well as a qualitative reaction. Had complement remained free or been added in excess of the amount necessary to act upon the bacterial cell, a certain amount of visible hemolysis must have occurred, and might, even, have been complete, had sufficient hemolytic amboceptor been present. These quantitative elements are the most important ones in the application of the test to be discussed later.

This test has been used for diagnostic purposes in many infectious diseases; for instance, in typhoid fever by Widal and Lesourd as well as by Hirschfeld, in cerebrospinal meningitis by Cohen, in whooping cough by Bordet and Gen-

gou, in scarlet fever by Besredka and Dopter and Foix and Mallein, in systemic gonococcic infections by Müller and Oppenheim, Bruck, Albarran and Jungano. Its most important and frequent application is in the sero-diagnosis of syphilis, as advanced by Wassermann, Neisser and Bruck and, independently, by Detre.

It is evident that the application of this complement fixation test leads, just as in the case of the agglutination and precipitin tests, to the identification of an antibody, provided one has the homologous antigen, or vice versa, the antigen and antibody necessarily being homologous and, hence, specific, the one for the other. It had been shown by Klebs, Metchnikoff and Roux that syphilis could be transmitted to monkeys by inoculation with human syphilitic material (antigen). Wassermann and his coworkers then found that the serum of these syphilitic apes contained an antibody not found in normal serum. Having this antibody, it was an easy step to show its relation to syphilitic antigen and to apply the complement fixation test in the detection of antibodies in the serum of patients infected with syphilis.¹ As the cultivation of the spirochete *Treponema pallidum* has only recently been achieved by Noguchi, no direct method of detecting these antibodies is available.² As indirect methods must be used, the antigen employed in the early studies was an extract of syphilitic tissues in the active stage of the disease, as these contain large numbers of the causative spirochete. Later workers have shown that this antigen need not be specific, as alcoholic extracts of certain normal organs, crude tissue lecithin, or, even, salts such as sodium glycocholate or oleate, may serve quite as well in the reaction. Although this fact lessens the true specificity of the test, from the biologic standpoint, there is no doubt of the clinical value of the reaction. The so-called syphilitic antigen must be either a normal substance or one possibly associated with an unknown product which actually produces a specific result. However this may be, it is a well established fact that, if the serum of a patient infected with syphilis be treated with antigen and complement, no hemolysis occurs on the later addition of hemolytic amboceptor and red cells because the complement is bound to the antigen through the medium of the homologous amboceptor; while if the patient is non-syphilitic marked hemolysis occurs as complement is free to act with the hemolytic system. This is the basis of the Complement Fixation Test as applied to syphilis by Wassermann and his colleagues. Although the antigen need not be specific, yet the test itself is remarkably specific being given by only a few non-syphilitic conditions as will be seen later.

In the actual technic of the test, many modifications have been advised, the variations consisting either in the antigen or in the cells to be hemolyzed or in both. Five essential factors enter into the reaction whatever be the modification employed. These are (1) syphilitic antigen, (2) syphilitic antibody, (3) erythrocytes (antigen), (4) hemolytic amboceptor and (5) complement. These factors must be accurately adjusted to one another, as serious errors may arise if the quantitative relations are not observed. The

¹ See Krauss, *Biochem. Ztschr.*, 1914, LXIV, 222.

² See foot-note on page 681 and 727.

writer will describe only the original Wassermann and the Noguchi methods, as these are, in his opinion, the most reliable ones to follow.¹

α. The Wassermann Reaction.

Preparation of Antigen.

Since the discovery of the fact that the antigen used in this test was not absolutely specific, in the biologic sense, for the syphilitic antibodies, many different antigens have been advocated. The writer selects those which have promised the best results, although he believes that Noguchi's antigen and, especially, Sachs' alcoholic heart antigen with cholesterin are by far the most reliable and constant ones introduced.

a. Aqueous Extracts.

1. Wassermann's Method.²

The liver or spleen of a syphilitic fetus is ground up in a mortar with sand or, preferably, minced in a meat grinder. The hash is placed in a dark-colored flask and mixed with 0.9 per cent. sodium chlorid solution containing 0.5 per cent. phenol. The proportion is 1 gram of substance to 4 c.c. of the salt solution. This mixture is shaken for 24 hours in a shaking machine and is then allowed to settle or is centrifuged. The reddish-brown opalescent supernatant liquid, which is the antigen, is decanted into dark bottles, tightly stoppered and kept on ice. On standing, a precipitate forms, which must not be disturbed by shaking. This aqueous antigen appears to be rather unstable and shows, sooner or later, some inhibition (anticomplementary action) with normal serum, although Citron³ finds such antigens active even after 11 months. Before use the activity of this extract, as well as of all others, must be quantitatively determined by the method later outlined. The usual doses are 0.2 (maximum) and 0.1 (minimum) c.c. As a general rule, we use only such an amount of antigen, the double dose of which shows no binding of complement.

b. Alcoholic Extracts.

1. Method of Porges and Meier.⁴

The liver of a syphilitic or normal fetus is hashed and extracted for 24 hours with five times its weight of absolute alcohol. Filter through filter-paper and distill the alcohol from the filtrate in vacuum at 40°C. A syrupy residue remains which is suspended in physiologic salt solution containing 0.5

¹ Hecht (Wien. klin. Wchnschr., 1909, XXII, 265) and later Weinberg (Ann. de l'Inst. Pasteur, 1912, XXVI, 427) utilize the natural amboceptor and complement present in unheated human serum. See, also, Gradwohl, Jour. Am. Med. Assn., 1914, LXIII, 240; Kolmer, Jour. Immunol., 1916, II, 23; Gradwohl, Jour. A. M. A., 1917, LXVIII, 514; Am. Jour. Syph., 1917, I, 450; Williamson, Jour. Lab. and Clin. Med., 1917, II, 658; Christian, Ibid., 1919, III, 613. Klauder and Kolmer (Jour. Am. Med. Assoc., 1921, LXXVI, 242) show that the Wassermann test applied to urine is of no value; they show, further (Ibid., 1923, V, 566) that this test is likewise of no value when performed upon milk, saliva, seminal fluid and aqueous fluid from the anterior chamber of the eye, but that exudates and transudates from syphilitics all yielded positive results, as did, also, surface fluid from chancres and saline extracts of syphilitic nodules. Graves (Ibid., 1920, LXXV, 592) believes that the Wassermann test performed upon blood collected post-mortem is as reliable as the ante-mortem test, 90.5 per cent. of cases showing post-mortem or clinical evidence of syphilis giving positive Wassermann tests.

² Deutsch. med. Wchnschr., 1906, XXXII, 745.

³ Die Technik der Bordet-Gengouschen Komplementbindungsmethode. in Handbuch of Kraus and Levaditi, Jena, 1909, p. 1076; Die Methoden der Immunodiagnostik und Immunotherapie, Leipzig, 1910. See, also, Bruck, Die Serodiagnose der Syphilis, Berlin, 1909.

⁴ Berl. klin. Wchnschr., 1908, XLV, 731.

per cent. phenol, using 100 parts of salt solution to 1 of syrup. Filter through filter-paper. The usual dose of this antigen is 0.2 to 0.3 c.c., although less quantities may be used as determined by methods of standardization.

Instead of this antigen, these workers use, also, a 1 per cent. solution of commercial lecithin in phenol-containing physiologic salt solution. This solution is stable if kept in the ice box. It has the disadvantage that the smaller doses may inhibit hemolysis, while the larger ones may, themselves, cause hemolysis. The stronger luetic sera require a larger dose (0.1 c.c.), while the weaker sera use less (0.01 c.c.) of lecithin. Hence we employ an average dose of 0.05 c.c., which may be compensated by the use of four to six times the titrated dose of amboceptor and complement.

2. Method of Landsteiner, Müller and Poetzel.¹

The muscular tissue, free from fat, of a guinea-pig heart is rubbed up in a mortar and extracted with 95 per cent. alcohol, using 50 c.c. for every gram of substance. Heat for several hours at 60° C. and filter through filter-paper. The filtrate is stable at room temperature for long periods. It is used in doses of 0.3 to 0.05 c.c.

3. Method of Sachs.²

As it had been shown³ that the addition of cholesterin to crude alcoholic extracts furnished an antigen of especially powerful and specific properties, although cholesterin itself had no antigenic effect, Sachs advocated a cholesterin-fortified extract of beef heart or liver or of guinea-pig heart. McIntosh and Fildes⁴ find that human heart furnishes a superior antigen. This latter material is used in the writer's laboratory, the antigen being prepared as follows:

The muscular tissue of a human heart, normal or pathologic, is freed from gross fat, finely minced, weighed, and ground up in a mortar with absolute alcohol in the proportion of 1 gram of tissue to 10 c.c. of absolute alcohol. Transfer the mixture to a stoppered bottle and shake for one and one-half hours at room temperature. Filter through paper and preserve in an amber stoppered bottle. Although the activity of this extract does not change for 2 or 3 months, a more reliable preparation may be had by preparing it fresh each month. To 5 parts of this 10 per cent. alcoholic extract of human heart add 4 parts of a 1 per cent. solution of cholesterin in absolute alcohol. This

¹ Wien. klin. Wchnschr., 1907, XX, 1565; 1908, XXI, 282; Ruediger (Jour. Infect. Dis. 1919, XXIV, 31 and 204) has shown that alcoholic extract of human heart, beef heart or rabbit heart give more reliable results than those extracts prepared from guinea pig heart.

² Berl. klin. Wchnschr., 1911, XLVIII, 2066.

³ See Browning, Cruickshank and McKenzie, Biochem. Ztschr., 1910, XXV, 85; Jour. Path. and Bacteriol., 1910, XIV, 484; Browning and McKenzie, Diagnosis and Treatment of Syphilis, Philadelphia, 1912; Desmoulière, Presse Méd., 1913, XXI, 898; Kolmer, Laubaug, Casselman and Williams, Arch. Int. Med., 1913, XII, 660; Thomas and Ivy, Jour. Am. Med. Assn., 1914, LXII, 363; Orkin, Berl. klin. Wchnschr., 1914, LI, 690; Thompson, Jour. Am. Med. Assn., 1914, LXII, 1458; Field, Ibid., 1620; Arch. Int. Med., 1914, XIII, 790; Judd, Jour. Am. Med. Assn., 1914, LXIII, 313; Owen and Snure, Jour. Mich. Med. Soc., 1914, XIII, 422; Interstate Med. Jour., 1914, XXI, 1281; Weston, Jour. Med. Research, 1914, XXX, 377; McClure, Ibid., 455; Walker, Arch. Int. Med., 1914, XIV, 563; Hopkins and Zimmermann, Am. Jour. Med. Sc., 1914, CXLVIII, 390; Kolmer and Schamberg, Ibid., 1915, CXLIX, 365; Cornwall, New York Med. Jour., 1915, CI, 844; Henes, Jour. Am. Med. Assn., 1915, LXIV, 1969; McClure and Lott, Am. Jour. Med. Sc., 1916, CLI, 712.

⁴ Ztschr. f. Chemotherap., Orig., 1912, I, 79.

forms the stock antigen. When used in the test a fresh 1 to 10 emulsion in normal saline is prepared by adding the stock antigen drop by drop with constant stirring to normal saline in proportion of 1 part of former to 9 of latter. Walker and Swift¹ believe that syphilitic patients under treatment should be treated until negative reactions obtain with a 1 to 6 dilution of this extract, as this gives a much more delicate reaction. The point to be remembered is that not more than one-fourth of the anti-complementary dose of this antigen should be used, although it is possible to use one-third of this dose providing very slight degrees of inhibition are disregarded and such tests called negative.

This antigen has the great advantage that its antigenic and anti-complementary properties are practically the same, although prepared from different hearts. The only criticism of this antigen is that it may yield non-specific fixation tests in a certain number of cases, but its greater sensitiveness enables it to catch a certain number of cases that are doubtful with other antigens. The tests run through uniformly and always show the same degree of activity, making the interpretation of the results a matter of greater accuracy. This antigen may be recommended as a standard one and should be used in every test made, even though any number of antigens be used with it.

c. Acetone-insoluble Extracts.

As it has been shown that the alcoholic extracts of various organs contain variable amounts of "syphilitic antigen," Noguchi² advocates the use of the alcohol and ether soluble but acetone insoluble extract of organs. This fraction contains lecithin and other phosphatids.

Method of Noguchi.

Extract a mashed paste of liver, heart, or kidney of man, ox, guinea-pig, rabbit, or dog with 10 parts of absolute alcohol at 37°C. for several days. Filter through filter-paper and collect the filtrate. The latter is then brought to dryness by evaporation with the aid of an electric fan or in a vacuum at 40°C. Take up the dried residue with ether and allow the turbid solution to stand in a covered dish over night in a cool place. Decant the clear ethereal solution into a clean beaker and concentrate by evaporating the most of the ether. Mix this concentrated ethereal solution with 10 volumes of pure acetone. Allow the precipitate to settle and decant off the supernatant fluid. This antigen is a light brownish precipitate which gradually becomes sticky on exposure to the air. This product usually has no hemolytic action upon human red cells, but may occasionally show this property. Further, it may, also, show an anticomplementary action. Three-tenths gram of this acetone-insoluble fraction is dissolved in 1 c.c. of ether and is mixed with 9 c.c. of methyl alcohol. If any precipitate forms or is left undissolved, remove it by centrifugation. This stock solution remains unaltered for a long period. For the actual test an aqueous emulsion is prepared by mixing 1 c.c. of the stock solution with 9 c.c. of physiologic salt solution. This produces a clear opalescent solution containing 0.3 per cent. of the original lipids. One-

¹ Jour. Exper. Med., 1913, XVIII, 75.

² Jour. Exper. Med., 1909, XI, 84; Serum Diagnosis of Syphilis, Philadelphia, 1912. See, also, L'Esperance and Coca, Jour. Immunol., 1916, I, 129; in the new homo-hemolytic system test of Noguchi, this antigen is the only one that may be reliably employed.

tenth c.c. of this emulsion is used in the test later described. This emulsion is kept on ice as it is unstable in this form.¹

Antigen Paper.—Instead of keeping a stock solution of antigen as above described, Noguchi has introduced the use of paper impregnated with antigen for the test. These papers are especially serviceable to the general worker who may wish to employ this test but they do not hold their properties longer than three months. These are far inferior to the liquid antigen but may be used in an emergency. Weigh out about 1.2 grams of the sticky extract and dissolve in about 20 c.c. of ether. Have ten sheets of filter-paper, 10 by 10 cm. in dimensions, laid one upon the other in a clean glass dish. Pour over these the lipid solution and saturate the paper evenly. Separate each sheet as quickly as possible and lay flat on a clean sheet of unbleached muslin. Within 10 minutes the paper is ready for use. Before assigning the dimensions for each tube in the fixation test, the antigen paper should be titrated. This is done as follows: Cut the paper into equal width, say 5 mm., and use increasing lengths of this strip for standardization, starting with 1 mm., 2 mm., etc. The principle of this standardization will be discussed later. The strips may be marked in sections, each representing the required dimensions, and put into sealed tubes for preservation.

The Syphilitic Antibody.

The immune serum may be withdrawn from the patient by the method given under blood cultures. The blood, thus obtained, is allowed to coagulate in a large test-tube. The serum is then drawn off with a pipet into small test-tubes and inactivated by heating to 56°C. for one-half hour.

In the writer's laboratory, the custom is to withdraw the blood from the median basilic vein by means of a pipet of about 20 c.c. capacity.² One end of this pipet is attached to a rubber tube with glass mouthpiece, while the other end bears a steel or platinum-iridium needle attached with rubber tubing. Five to ten c.c. of blood are withdrawn and placed immediately in a small test-tube where it is allowed to clot. After about 20 minutes, the clot is separated from the walls of the tube and the tube is then centrifuged to obtain the clear serum. If the specimen is drawn by the physician and is to be forwarded to the laboratory, it is essential that the cells be separated from the serum before the specimen can be sent.³ Such specimens give reliable results, but if the cells are allowed to remain in contact with the serum for any length of time, so much hemolysis occurs that the test can not be accurately made by a distant

¹ Noguchi has recently (Jour. Am. Med. Assn., 1912, LVIII, 1163) prepared a specific antigen from cultures of the *treponema pallidum*. The most interesting finding, based upon comparative studies with this antigen and with the lipid antigens, is that there is no indication, in cases of active syphilitic orchitis, of a sufficient amount of specific antibodies to bind complement with the pallida antigen, although a strong positive Wassermann reaction obtains. See Craig and Nichols, Jour. Exper. Med., 1912, XVI, 336; Kolmer, Williams and Laubaug, Jour. Med. Research, 1913, XXVIII, 345; Varney and Baeslack, Jour. Am. Med. Assn., 1913, LXI, 754.

² See Parvu, Arch. des Mal. du Cœur, 1915, VIII, 439.

³ Ruediger (Phil. Jour. of Sc., 1917, XI, 1 and 87) states that glycerin keeps the serum sterile and does not noticeably influence the reaction. Hence this may be used in cases where it is necessary to delay the test for some time.

laboratory. The serum is inactivated by heating to 56°C. for one-half hour either immediately after centrifugation or on receipt of the specimen.

This inactivation is absolutely essential provided aqueous or alcoholic extracts are used as antigens, because these antigens contain various proteins, which are liable to give non-specific proteotropic fixation with active sera. Noguchi has shown that this property is lost if the serum be inactivated. Moreover, with his antigen (acetone-insoluble lipoids) no such proteotropic fixation occurs, so that active sera may be employed in his test, although the inactivated serum works quite as well.

Suspension of Red Cells.

In the Wassermann test, the corpuscles to be hemolyzed are those of the sheep. The blood may be obtained from an abbatoir or sheep may be kept in the animal rooms of the laboratory and bled from the ear as desired. The blood is received directly in a more than equal amount of 1 per cent. sodium citrate solution in physiologic salt solution or it may be defibrinated by shaking with glass beads or by whipping with wires or bristles. It is then centrifuged and the supernatant fluid drawn off. The cells are thoroughly mixed with physiologic salt solution, again centrifuged and the clear fluid withdrawn. This process is repeated two or three times until the corpuscles are free from serum. The sedimented corpuscles are now drawn up with a graduated pipet and added to 19 volumes of salt solution. A 5 per cent. suspension is thus obtained, of which 1 c.c. is used in the test. If the centrifuge tube be calibrated, one may use this directly in making the suspension. Such suspensions may be used for two or three days, especially if kept in the ice box, but they should be discarded if any trace of hemolysis appears in the tube.¹

Hemolytic Amboceptor.

The hemolytic serum is obtained from rabbits which have been injected with washed sheep corpuscles. If the blood is received from the abbatoir, it is usually contaminated to such an extent that it must be sterilized by heating to 60°C. for one-half hour. The cells are obtained as above described and washed very thoroughly with salt solution. When free from serum, their volume is made up to that of the original defibrinated blood with physiologic salt solution. The immunization is best carried out by injecting a rabbit, intraperitoneally, with 2, 4, 8, and 12 c.c. of cells at intervals of 4 or 5 days. Ten days after the last injection a few c.c. of blood are obtained from an ear vein of the rabbit and the serum tested for its hemolytic effect upon sheep corpuscles. If it produces hemolysis in dilutions of 1 to 600 or more, the animal is bled from the carotid artery, the blood being collected in a series of sterile test-tubes. These are placed in the ice box, the blood allowed to coagulate and the serum is drawn off. This serum is then inactivated and put into small sterile tubes, which should be sealed and kept on ice. This material keeps well, but its strength must be determined at frequent intervals.

¹ See Trossarello (Rif. Med., 1914, XXX, 95) also, Rous and Turner (Proc. Soc. Exper. Biol. and Med., 1915, XII, 122, Jour. Exper. Med., 1916, XXIII, 219 and 230) for methods of preserving these blood cells. Reimann, Jour. Lab. and Clin. Med., 1917, II, 200; Dreyer and Gardner, Lancet, 1919, II, 687.

It is wise to inject several animals at the same time, so that one may surely yield a satisfactory serum of high hemolytic power.¹

Complement.

Normal guinea-pig serum is used as complement in most all modifications of this test.² The animal is anesthetized and the blood drawn from the carotid, as above described, into sterile test-tubes in which it is allowed to coagulate. The serum is then drawn off with a sterile pipet, placed in small sterile test-tubes and kept on ice.

As complement deteriorates rapidly (being of little use after 48 hours) the blood is, preferably, withdrawn by aspiration directly from the heart.³ The animal will usually survive the loss of 5 to 10 c.c. of blood, providing the heart has not been badly lacerated by repeated punctures. After two weeks the animal may be used for a fresh supply of complement. It is wise to have quite a number of these animals on hand so that fresh material may constantly be available. It is absolutely unnecessary to sacrifice an animal each time a few tests are to be made. When used complement is diluted 1 to 10 with physiologic salt solution.⁴

Standardization of Reagents.

This standardization must always be preliminary to the actual performance of the test. Everything depends upon the proper adjustment of the various factors entering into the test, so that especial care must be taken to find out just what quantities of each reagent to use. Without such precautions, little, if any, value can be attached to any report.

Titration of Hemolytic Amboceptor.

Prepare a series of dilutions of the inactivated serum beginning with 1 to 300 and extending to 1 to 3,000. This may be readily accomplished by diluting 0.1 c.c. of serum to 30 c.c. with physiologic salt solution and using this 1 to 300 dilution as the basis of further dilutions. Arrange a series of small test-tubes and place in each 0.25 c.c. of these diluted sera, beginning with the highest concentration and extending to the lowest. Now add 0.25 c.c. of a 1 to 10 dilution of complement and 0.25 c.c. of a 5 per cent. suspension of sheep corpuscles to each tube. Bring the total volume up to 1.25 c.c. with physiologic salt solution. All tubes are placed in the incubator at 37°C. for

¹ See Coca, *Jour. Infect. Dis.*, 1915, XVII, 361; also, Morgenroth and Bieling, *Biochem. Ztschr.*, 1915, LXVIII, 85.

² Noguchi (*Jour. Exper. Med.*, 1918, XXVIII, 43) has introduced a new modification of his test using a homohemolytic system by the employment of human complement. See, also, Lewis and Newcomer, *Ibid.*, 1919, XXIX, 351; Bruynoghe, *Bull. acad. roy. med. Belg.*, 1919, XXIX, 209, 222, and 231; Golay, *Rev. Méd. de la Suisse Rom.*, 1919, XXXIX, 493.

³ See Austin (*Jour. Am. Med. Assn.*, 1914, LXII, 868) and Weston (*Jour. Med. Research*, 1915, XXXII, 391) for methods of preserving complement; Thompson, *Jour. A. M. A.*, 1916, LXVI, 652; Rhamy (*Jour. A. M. A.*, 1917, LXIX, 973) advises the use of sodium acetate as a preservative, mixing 4 parts of complement with 6 parts of a 10 per cent. solution of sodium acetate in 0.9 per cent. sodium chloride solution. Emerson (*Jour. Lab. and Clin. Med.*, 1919, V, 62) advocates the use of chloroform.

⁴ See Tissot (*Compt. rend. Acad. de Sc.*, 1914, CLVIII, 1525); Thorsch (*Biochem. Ztschr.*, 1915, LXVIII, 67); Ottenberg, Reuben and Frazier (*Jour. Infect. Dis.*, 1915, XVI, 119) for studies of the nature of complement. Cole (*Cleveland Med. Jour.*, 1915, XIV, 273) show the presence of anti-sheep amboceptor in guinea-pig's blood—Müller, *Wien. klin. Wehnschr.*, 1916, XXIX, 1239; Ruediger, *Jour. Infect. Dis.*, 1919, XXIV, 120.

two hours and are then allowed to stand over night in the ice box. The highest dilution (smallest amount of amboceptor) in which complete solution of the cells occurs is taken as the strength of the serum. This is called one amboceptor unit. In performing the Wassermann test, two units of amboceptor are used. The titer of the amboceptor remains fairly constant, but it is wise invariably to determine its value when fresh complement is to be used, owing to the marked influence of these factors on one another.

Instead of using the above method of standardization, one may resort to the drop method of Landsteiner, Müller and Poetzl, which simplifies the technique but, in so doing, impairs the accuracy of the result, in the writer's opinion, as the drops from different capillary or other pipets are seldom of the same size. By this method one determines what dilution of amboceptor in 1 drop dose will hemolyze in one-half hour, in an incubator, 1 drop of 50 per cent. suspension of sheep's corpuscles with 1 drop of complement, 10 drops of salt solution being added as diluent. Twice the strength of the titer is used in the test.

Titration of Complement.

Having obtained the value of the amboceptor unit, one may use decreasing amounts of complement with one unit of amboceptor in the same way as in the above titration, the dilution of the complement running from 1 to 2 to 1 to 20. Complement is usually used in a dilution of 1 to 10, 1 c.c. being employed in the test. This is, however, open to objections as it is not absolutely quantitative. Noguchi has pointed out the fact that variation in the amount of complement used influences the amount of amboceptor. "If less than one unit of amboceptor is used hemolysis will always be incomplete, even with more than one unit of complement. Likewise, if with one amboceptor unit there is combined less than one unit of complement, hemolysis can not be complete. If with more than one unit of amboceptor there be used less than one unit of complement, hemolysis may be complete or incomplete according to the relative amounts of each factor used.¹ In the presence of many units of amboceptor hemolysis may be complete when but a small fraction of the complement unit is present."

Titration of Antigen.

The degree of dilution in which the antigen is to be used must be determined for each extract by testing it both against normal and syphilitic sera. Its concentration must be such that it does not prevent hemolysis with normal sera, while it will promptly inhibit hemolysis in presence of complement with syphilitic sera.²

Set up two parallel series of tubes. Into each tube of one series place 0.25 c.c. of a 1 to 5 dilution of known syphilitic serum and into each tube of the second series place 0.25 c.c. of a 1 to 5 dilution of normal serum. Dilutions of the antigen are now made, with physiologic salt solution as the diluent, begin-

¹ See Kromayer and Trinchese, *Med. Klin.*, 1912, VIII, 404 and 1670, who use an absolute minimum of complement in their "refined" Wassermann test; also, Thompson, *Arch. Int. Med.*, 1914, XIII, 904.

² See Ottenberg, *Jour. Immunol.*, 1916, II, 47; Denzer, *Jour. Infect. Dis.*, 1916, XVIII, 631.

ning with 1 to 1 and running up to 1 to 20 or higher if necessary. Into the first tubes of the above series place 0.25 c.c. of the first antigen dilution, into the second tubes of each series place the second dilutions and so on until all are accounted for. To all of the above tubes add 0.25 c.c. of complement (1 to 10) and incubate at 37°C. for one hour. Now add 0.25 c.c. of hemolytic amboceptor in twice the maximum dilution found to cause complete hemolysis (two units) and 0.25 c.c. of a 5 per cent. suspension of sheep corpuscles. Place in an incubator for two hours and then in ice box over night. No hemolysis should be observed in the tubes containing syphilitic serum except in those containing the higher dilutions, while in the tubes with normal serum hemolysis will occur in all tubes except those with the lower dilutions of antigen. Hemolysis in the tubes with syphilitic sera is due to the normal hemolytic power of the antigen, while the lack of hemolysis in the tubes with normal sera is due to the anticomplementary action of the antigen. In carrying out the Wassermann test, antigen is used in one-half the lowest dilution (largest amount of antigen) which gives complete hemolysis with normal sera, this being taken as one antigen unit. Thus if a dilution of 1 to 5 gave complete hemolysis, a dilution of 1 to 10 would be used in the actual test.

If one uses the drop method, as mentioned above, 1 drop of antigen (undiluted) and 1 drop of complement (undiluted) are mixed with 10 drops of salt solution. The mixture is incubated one-half hour and 1 drop of amboceptor and 1 drop of a 50 per cent. suspension of corpuscles are added. Hemolysis should occur within one-half hour. If it does not, anticomplementary action is marked and less antigen must be employed in the test.

Having determined the strength of the antigen, a portion of the stock solution is diluted with salt solution so that 1 c.c. equals one unit. In testing the antigenic properties of this antigen, one may use it with a large number of positive and normal controls, or, preferably, run it in parallel series with an antigen of known value. If the results agree, it may then be substituted for the old antigen. The titer being known, the antigen may be used for subsequent tests without redetermination of this value.

Technic of the Test.

Original Wassermann Method.

All of the glassware used in the test must be kept thoroughly clean and dry, but need not be sterile. The pipets, which should be both graduated to 0.01 and 0.1 c.c., should, preferably, be used with only one reagent. If they are employed with others, they must be thoroughly rinsed in salt solution between such applications. After using, wash them well in salt solution (never with soap and water), allow them to stand in a tall cylinder of distilled water for a few hours and dry them in an oven. The test-tubes used in this test are about 14 cm. in length by 1.5 cm. in diameter, while the somewhat smaller ones (1 by 10 cm.) may be employed in the Noguchi modification.

Before actually performing the test, the reagents are prepared and standardized as previously described or the amboceptor may be titrated at the beginning of the test, as it is not added until the preliminary incubation of the serum with the antigen and complement. The sera of the patient and of the

known positive and normal controls must all be inactivated by heating to 56°C. for one-half hour.¹ The complement is diluted 1 to 10 with physiologic salt solution and the suspension of washed sheep corpuscles prepared. When these preliminaries are attended to one may proceed with the test itself.

Arrange a double row of test-tubes, the front tube of each set to receive the antigen and the back row the control tube without antigen. One set of tubes must be placed for the unknown serum, one set for the positive and one set for the normal control; while other controls are arranged as spoken of later.

1. Into both tubes of each set place 0.2 c.c. of the serum to be tested.
2. To all tubes, add 1 c.c. of complement (diluted 1 to 10).
3. To tubes in front row only, add 0.2 c.c. of antigen (aqueous extract of syphilitic liver).

Make up volume of each tube to 3 c.c. with salt solution, mix contents by shaking and incubate at 37°C. for one hour.²

4. Add to each tube 1 c.c. of antishoop amboceptor (two units).
5. Add to each tube 1 c.c. of 5 per cent. suspension of sheep corpuscles.

Mix the contents of the tube thoroughly, incubate at 37°C. for two hours and place the tubes in the ice box over night. At the end of this period the results are read.

Controls.

1. Positive syphilitic serum. To be run as above.
2. Known negative serum. To be run as above.
3. A tube containing complement and two units of antigen, to prove that no anticomplementary action of the antigen obtains.
4. A tube containing complement, to show that the hemolytic system is active.
5. A tube containing antigen alone, to show absence of lytic power of antigen.

All controls are brought up to 3 c.c., incubated and, then, amboceptor and sheep corpuscles are added as above.

If the test is properly conducted, hemolysis will occur in every control tube excepting in the tube containing syphilitic serum and syphilitic antigen (the positive control) and in the tube of control 5. In the series of unknown sera,

¹ Gramenitzki (Biochem. Ztschr., 1912, XXXVIII, 501) and Fenyvessy (Ibid., 1912, XL, 353) have shown that if the serum be not heated to complete inactivation, the complement is restored on allowing to stand for some time.

² Recent work has shown that a lower temperature of the first incubation yields a higher percentage of positive results in known positive cases and, also, that this method, catches more of the less easily recognized cases than does the usual incubation at 37°C. Smith and MacNeal (Jour. Immunol., 1916, II, 75; Jour. Infect. Dis., 1917, XXI, 233) advocate incubation in the refrigerator at approximately 8°C. for 4 to 24 hours. Dean (Jour. Path. and Bact., 1917, XXI, 193) and Ruediger (Jour. Infect. Dis., 1918, XXIII, 173; 1919, XXIV, 405) confirm these results. Noguchi (Jour. Exper. Med., 1918, XXVIII, 297) maintains that the optimum temperature is 37°C. but that maximum reactions may be reached at temperatures not lower than 23°C. Noguchi's results apply only to the test in which acetone-insoluble antigen is used, although the results of Smith and MacNeal show that fixation with this antigen is more specific by the "ice box method." See, also, Berghausen, Jour. A. M. A., 1919, LXXII, 996; Hitchcock, U. S. Naval Med. Bull., 1919, XIII, 740; Wile and Hasley, Jour. A. M. A., 1919, LXXII, 1526; Owen and Martin, Jour. Lab. and Clin. Med., 1920, V, 232; Mackie and Rowland, Brit. Jour. Exp. Path., 1920, I, 219; Berghausen, Jour. Am. Med. Assoc., 1920, LXXIV, 1166; Smith, Philippine Jour. Sc., 1920, XVII, 31; Owen and Martin, Jour. Lab. & Clin. Med., 1920, V, 232; Rhamy, Am. Jour. Syph., 1921, V, 300; Duke, Ibid., 312; Jour. Lab. & Clin. Med., 1921, VI, 392; McIntyre, Worth and McIntyre, Ibid., 706; Wyler, Jour. Path. & Bact., 1921, XXIV, 340; Keidel and Moore, Bull. Johns Hopk. Hosp., 1921, XXXII, 296.

no hemolysis will occur in the tubes containing syphilitic serum. This lack of hemolysis, partial or complete, is, of course, a positive reaction.

Citron¹ advises the use of two tubes for serum and antigen, one tube containing 0.2 c.c. of serum and 0.2 c.c. of antigen as given above, while the other tube contains 0.1 c.c. of serum and 0.1 c.c. of antigen. In this way the results may be represented somewhat more quantitatively, as the degree of inhibition of hemolysis varies according to the amount of syphilitic antibody present. If this amount be large, complete inhibition occurs in the tube containing 0.1 c.c. of serum and 0.1 c.c. of antigen as well as in tube No. 1, the result being expressed as + + + +. If inhibition is incomplete in tube 2 but complete in tube 1, the result is + + +. Both of these degrees are strongly positive reactions. If tube 2 is completely hemolyzed while tube 1 is completely inhibited, the result is + +. Incomplete inhibition in tube 1 and complete hemolysis in tube 2 is expressed as +. These two latter results are classed as weakly positive reactions. If tube 1 shows doubtful inhibition while tube 2 shows complete hemolysis, the result is \pm . Complete hemolysis in both tubes is, of course, a negative result.²

Noguchi's Modification.

This is not to be confused with Noguchi's test in which a human hemolytic system is used. The modification here suggested uses a different antigen and different quantities of the same reagents as employed by Wassermann. This is the method, followed in the writer's laboratory, when the sheep hemolytic system is used, a control being run with Sachs' antigen or a specific watery extract as antigen in cases showing affections of the central nervous system.

1. Set up the double row of tubes as in the preceding test using 0.2 and 0.1 c.c. of serum to be tested.

2. To all tubes add 0.1 c.c. of an undiluted complement.

3. To front row only, add 0.1 c.c. of acetone-insoluble antigen.

Make up volume of each tube to 1.5 c.c. with physiologic salt solution. Mix contents thoroughly and incubate at 37°C. for one hour.

4. Add to each tube 1 c.c. of antishoop amboceptor (two units).

5. Add to each tube 0.5 c.c. of a 10 per cent. suspension of sheep corpuscles.

The total volume is now 3 c.c. Mix contents thoroughly and incubate at 37°C. for two hours. Allow to stand four to six hours at room temperature and read results. The interpretation is as given above.

Drop Method.

This is the method of Landsteiner, Müller and Poetzl and is frequently employed. The writer can not convince himself that this method is an accurate one owing to the possible variations in size of the drops, which are delivered from a capillary pipet.

¹ Handbook of Kraus and Levaditi, 1908, II, 1105.

² Ivy (Jour. Am. Med. Assn., 1912, LIX, 432) advocates the use of the Duboscq colorimeter as a means of estimating the exact degree of hemolysis, the control tube of each case being taken as the standard of 100 per cent. Ninety per cent. and over of hemolysis is regarded as negative. See Kaplan (New York Med. Jour., 1913, XCVII, 1172; Ibid., 1913, XCVIII, 157); and Kaplan and McClelland (Ibid., 1912) for the determination of amino nitrogen as a control.

1. Set up the double row of tubes as in the preceding tests and add to each 10 drops of physiologic salt solution.
 2. Add 1 drop of serum to each of the two tubes used for the sera tested.
 3. To front row only, add 2 drops of antigen.
 4. To all tubes add 1 drop of complement.
- Mix thoroughly and incubate for one hour at 37°C.
5. Add 1 drop (two units) of standardized antisheep amboceptor to each tube.
 6. Add 1 drop of a 50 per cent. suspension of sheep corpuscles to each tube.
- Incubate for two hours. Allow to stand at room temperature and read results. The method of interpretation is as above.¹

Method of Noguchi.²

It has been found that human serum contains a variable amount of natural antisheep amboceptor.³ This, of course, increases the effect of the immune antisheep amboceptor used in the tests previously described. One may, therefore, obtain a negative Wassermann test under these circumstances even though a large amount of complement is fixed. Noguchi has overcome this possible error by introduction of a human hemolytic system to replace the sheep hemolytic system of the original test. He employs, also, a smaller amount of reagents and uses either fresh or inactivated serum of the patient. If preferred, one may use larger quantities of patient's serum, than outlined by Noguchi; if so, the reagents are increased in the same proportion. This method of Noguchi is the one preferred by the writer and is the one followed as a routine in his laboratory, as it has been found somewhat more reliable than the original Wassermann test in obscure cases.⁴ Sachs' antigen is used in every test.

Collection of Serum.

Noguchi obtains the patient's blood from a puncture on the ventral side of the last joint of the middle finger. The finger may be compressed by squeezing before the puncture and, afterward, massaged to press out the blood, this being collected in small tubes drawn out into capillary points. In the writer's laboratory the blood is obtained as given on page 727.

Corpuscle Suspension.

The suspension may be prepared with the blood of the patient or with that of the examiner. If with that of the former, it is especially important that every trace of serum be removed by careful washing. The standard amount of corpuscle suspension is 1 c.c. of a 1 per cent. or 0.1 c.c. of a 10 per cent. suspension for each tube. If the patient's blood is to be used, enough may be obtained for many tests as follows: Fill a graduated centrifuge tube to 9 c.c.

¹ See Mefford, *Illinois Med. Jour.*, 1914, XXVI, 428.

² *Jour. Exper. Med.*, 1909, XI, 392; *Serum Diagnosis of Syphilis*, Phila., 1912.

³ See Dexter and Cummer, *Arch. Int. Med.*, 1912, IX, 605; also, Bailey, *Ibid.*, 551.

⁴ See Coca and L'Esperance (*Arch. Int. Med.*, 1913, XI, 84) for a slight modification of the Noguchi technic. See, also, Miller, *Interstate Med. Jour.*, 1913, XX, 145; Thompson, *Arch. Int. Med.*, 1913, XI, 512. See Cecil and Lamb (*Arch. Int. Med.*, 1913, XI, 249) for the value of this test in testing the serum of cadavers.

with 2 per cent. sodium citrate in physiological salt solution. Allow the blood to drop in until it fills the tube to 10 c.c. Mix and centrifuge. Pour off the supernatant fluid and fill up to 10 c.c. with fresh salt solution. Mix thoroughly and again centrifuge. This may be repeated two or three times, in order to insure removal of every trace of serum. The deposited corpuscles may now be suspended either in 100 c.c. of salt solution (making a 1 per cent. suspension) or in 10 c.c. (giving a 10 per cent. suspension). This procedure, while thoroughly reliable, is not followed by the writer. It is our custom to obtain the corpuscles from a puncture of the finger of the worker, allowing 3 drops of blood to fall into 12 c.c. of salt solution in a centrifuge tube. This gives, approximately, a 1 per cent. suspension. The washing is done as above. This latter method enables us to obtain fresh suspensions whenever desired.

Hemolytic Amboceptor.

This is prepared, as previously outlined, by injecting a rabbit, intraperitoneally, with increasing doses of washed human corpuscles, instead of with sheep corpuscles as in the Wassermann method. After the animal is immunized, the blood is collected and allowed to coagulate. The serum is then withdrawn and titrated as previously described.

Amboceptor Paper.—This has been introduced by Noguchi to simplify the test and to enable those not in close touch with clinical laboratory facilities to perform the test. While this paper may be used, the writer advises those doing any number of these tests to place their reliance more in the liquid amboceptor. The serum, obtained as above, is poured over sheets of filter-paper (Schleicher and Schüll's No. 597), cut into squares of 10 x 10 cm. Allow all the sheets to become evenly wet and absorb the excess with another sheet of paper. These are then dried at room temperature by placing each square separately upon a clean sheet of unbleached muslin. When dry, the sheets are cut into strips 5 mm. wide and are then standardized as follows: Take a series of tubes containing 1 c.c. of the 1 per cent. erythrocyte suspension and add to each tube 0.02 c.c. (as one unit) of complement. Now add the strips in increasing lengths, as 1 mm., 2 mm., 3 mm., etc., and incubate for two hours. The shortest strip causing complete hemolysis in this time represents one unit of amboceptor. These strips are then marked into sections of twice this length (two units) and cut off when the actual test is to be made. The papers should be kept dry and sealed. The papers on the market are quite variable and are not always to be relied upon. As previously stated, these papers should be used only when the liquid amboceptor is not at hand.

Complement.

Guinea-pig serum is used as complement in this system as in the others. The dilution is 40 per cent. made by mixing 1 c.c. of complement with $1\frac{1}{2}$ c.c. of physiologic salt solution. The method of obtaining it has been given above. Complement paper may be prepared but it is very unsatisfactory and should not be used if the liquid form can possibly be obtained.

ORIGINAL WASSERMANN TEST

	Human serum	Complement (guinea-pig serum 1 to 10)	Antigen		Antisheep amboceptor two units	Five per cent. suspension of sheep corpuscles		Hemolysis
	C.C.	C.C.	C.C.	Make up volume of each tube to 3 c.c. with salt solution. Mix contents and incubate at 37° one hour	C.C.	C.C.		
Unknown serum	0.2	I	0.2		I	I		Depends on sera.
Unknown serum	0.2	I		I	I		Complete.
Positive control.....	0.2	I	0.2		I	I		None.
Positive control.....	0.2	I		I	I		Complete.
Negative control.....	0.2	I	0.2		I	I		Complete.
Negative control.....	0.2	I		I	I		Complete.
Anticomplementary control.	I	0.4		I	I		Complete.
Hemolytic control.....	I		I	I		Complete.
Antigenic control.....	0.2		I	I		None.
							Mix. Incubate at 37° for two hours. Allow to stand in ice box over night. Read	

NOGUCHI'S MODIFICATION (SHEEP HEMOLYTIC SYSTEM)

	Human serum	Complement (guinea-pig serum undil.)	Antigen		Ten per cent. suspension of sheep corpuscles	Antisheep amboceptor. Two units		Hemolysis
	C.C.	C.C.	C.C.	Make up volume of each tube to 1.5 c.c. with salt solution. Mix contents and incubate at 37° for one hour	C.C.	C.C.		
Unknown serum ...	0.2	0.1	0.1		0.5	I		Depends on sera.
Unknown serum	0.2	0.1		0.5	I		Complete.
Unknown serum	0.1	0.1	0.1		0.5	I		Depends on sera.
Unknown serum	0.1	0.1		0.5	I		Complete.
Positive control.....	0.1	0.1	0.1		0.5	I		None.
Positive control.....	0.1	0.1		0.5	I		Complete.
Negative control....	0.2	0.1	0.1		0.5	I		Complete.
Negative control....	0.2	0.1		0.5	I		Complete.
Anticomplementary control.	0.1	0.2		0.5	I		Complete.
Anticomplementary control.	0.1	0.1		0.5	I		Complete.
Hemolytic control...	0.1		0.5	I		Complete.
							Mix. Incubate at 37° for two hours. stand four to six hours at room temp. Read	

NOGUCHI'S MODIFICATION (HUMAN HEMOLYTIC SYSTEM)

	Human serum	Complement (guinea-pig serum 1 to 40)	Antigen	One per cent. suspension of human corpuscles		Antihuman amboceptor		Hemolysis
	C.C.	C.C.	C.C.	C.C.	Mix thoroughly and incubate for one hour at 37°			
Unknown serum....	0.02	0.1	0.1	I		2 units		Depends on sera.
Unknown serum....	0.02	0.1	I		2 units		Complete.
Positive control.....	0.02	0.1	0.1	I		2 units		None.
Positive control.....	0.02	0.1	I		2 units		Complete.
Negative control....	0.02	0.1	0.1	I		2 units		Complete.
Negative control....	0.02	0.1	I		2 units		Complete.
Anticomplementary control.	0.1	0.1	I		2 units		Complete.
Hemolytic control...	0.1	I		2 units		Complete.
							Mix and incubate at 37° for two hours. Allow to stand at room temp. for few hours. Read	

Antigen.

The antigen, used in this system, is prepared as previously described (p. 726). The stock solution is a 3 per cent. methyl alcohol solution of the acetone-insoluble lipoids. From this a 0.3 per cent. emulsion is prepared for the test by mixing 1 c.c. of this stock solution with 9 c.c. of physiologic salt solution. It seems advisable always to control this antigen by running a control test with a watery extract of specific organs, and of Sachs' antigen, especially if an affection of the central nervous system obtains.

Technic.

1. Arrange the double row of tubes with same controls as in the other tests. Place in all tubes 1 capillary drop (0.02 c.c.) of the fresh serum to be tested. If the serum has been inactivated use 4 drops (0.08 c.c.). One may use 0.2 c.c. of fresh (not inactivated) cerebrospinal fluid.

2. Add to each tube 0.1 c.c. of 40 per cent. complement, or in a great emergency two units of complement paper.

3. To the tubes in front row of each set, add 0.1 c.c. of antigen emulsion.

4. To both tubes of each set, add 1 c.c. of 1 per cent. washed human corpuscles.

Mix thoroughly and incubate for one hour at 37°C.

5. Add to each tube 2 units of antihuman amboceptor in liquid or paper form.

Mix and incubate for two hours at 37°C. Allow the tubes to stand at room temperature for a few hours and read results.

The results of this test are interpreted as in the preceding ones. This human system is more adjustable and reliable than is the sheep system. Noguchi has pointed out certain variable factors which may obtain and which may require a proper adjustment to insure proper results. These variables are, it is to be understood, greater in the sheep system, and are far less easily controlled. I quote directly from Noguchi. (1) One sometimes meets with instances in which hemolysis is complete within 10 to 20 minutes, and in which the positive control tubes with antigen undergo, sooner or later, gradual hemolysis. Such rapid progress of hemolysis at first mentioned is a sign of imperfect reaction. If the test is properly made, hemolysis proceeds gradually, and is complete in the water-bath within half an hour or thereabout. The causes of this accelerated hemolytic process are either an abnormally weak resistance of the blood corpuscles, or an exceptionally high activity or insensitiveness to fixation of the complement employed; or it may be the result of all these acting together.¹ It happens occasionally that the serum of certain guinea-pigs contains an abnormally active complement. In order to establish this point, and thus to remove this source of error, one has only to make the test with a quantity of complement which corresponds exactly to two complement units. (2) There are sometimes encountered instances in which hemolysis remains incomplete even in the control tubes in which there is no antigen. Here the causes of the imperfect reaction are found either in the weakness of the complement, or the amboceptor used, or both.² Usually

¹ See Bailey, *Jour. Exper. Med.*, 1912, XV, 470.

² See Barratt, *Jour. Path. and Bacteriol.*, 1912, XVI, 363.

the cause is the weakness of the complement, which, owing to its great lability, is likely to deteriorate. The activity of the amboceptor is far less subject to external influences which bring about its deterioration, and it is therefore extremely rare to find that the imperfection in the reaction arises from this source. In testing several specimens of serum at one time it happens occasionally that some specimens are slower in completing the hemolytic reaction than others. The cause of this slowness is not present in the complement or amboceptor, but in the specimens themselves.¹ In such cases the specimens are found to contain anticomplementary substances which react with and reduce the activity of the complement. To remove this source of error, it is necessary to heat the serum to 55°C. for 20 minutes and use four drops for the test. The difficulty may be obviated in some cases by collecting specimens of serum to be tested just before meal-time because the anticomplementary substance is closely associated with the absorption of the chyle into the circulation soon after the meal. (3) The quality and quantity of the antigen can also be sources of error. If one uses poor antigen, either there will be no positive reaction at all, or weak positive reactions will be entirely overlooked. If, on the other hand, an excessive amount of unfractionated crude antigen is employed, certain nonspecific weak reactions may become manifest, or a false positive reaction even may be obtained, as the result of the action of anticomplementary substances sometimes contained in preparations of the antigen. These sources of error can be entirely excluded by choosing an antigen that has been carefully prepared and standardized.²

¹ See Bronfenbrenner and Noguchi, *Jour. Exper. Med.*, 1912, XV, 598 and 625; Olson (*Jour. Lab. and Clin. Med.*, 1916, I, 704) calls attention to the importance of what he styles this "Delayed Wassermann Reaction." McConnell (*Ibid.*, 1919, V, 43) studies this reaction by examining the tubes every 15 minutes after placing the tubes, to which the hemolytic system was added, in a water bath at 37°C. He interprets as a "delayed" reaction, one in which the tube containing cholesterinized antigen showed no hemolysis at the end of 30 minutes but had completely cleared up at the next period of examination. No such delay is observed in the tube containing plain alcoholic antigen. About 1 per cent. of all Wassermann tests show this delayed reaction.

² For discussions of many obscure points regarding the nature of the reagents and the technic of the test see Addis, *Jour. Infect. Dis.*, 1912, X, 200; Dick, *Ibid.*, 1913, XII, 111; Lippmann and Plesch, *Ztschr. f. Immunitätsforsch.*, 1913, XVII, 548; Baumann, *Illinois Med. Jour.*, 1913, XXIV, 117; Burdick, *Colorado Med.*, 1913, X, 135; Kolmer and Casselmann, *Jour. Med. Research*, 1913, XXVIII, 369; Surface and Routt, *Ibid.*, 441; Cummins, *Ibid.*, 1913, XXIX, 23; Weil, *Biochem. Ztschr.*, 1913, XLVIII, 347; Stillians, *Jour. Cutan. Dis.*, 1913, XXXI, 316; von Gierke, *Deutsch. med. Wchnschr.*, 1913, XXXIX, 502; Rabinowitsch, *Ibid.*, 1210; Kolmer and Williams, *Jour. Infect. Dis.*, 1913, XIII, 60; Spät, *Biochem. Ztschr.*, 1913, LVI, 21; Kapsenberg, *Nederl. Tijdschr. v. Geneesk.*, 1913, II, 1007; Wassermann and Lange, *Kolle und Wassermann's Handb.*, 1913, VII, 951; Lesser, *Münch. Med. Wchnschr.*, 1914, LXI, 70; Langer, *Deutsch. med. Wchnschr.*, 1914, XL, 273; Olmstead, *Med. Record*, 1914, LXXXV, 341; Thiele and Embleton, *Lancet*, 1914, I, 520; McIntosh and Fildes, *Ibid.*, 860; Matzenauer and Hesse, *Wien, klin. Wchnschr.*, 1914, XXVII, 319; Sternberg, *Ibid.*, 545; Klein and Fränkel, *Münch. med. Wchnschr.*, 1914, LXI, 651; Wechselmann, *Berl. klin. Wchnschr.*, 1914, LI, 304; Blumenthal, *Ibid.*, 1310; Nathan, *Ibid.*, 1934; Stone, *New York Med. Jour.*, 1914, XCIX, 1242; Grulec, *Am. Jour. Med. Sc.*, 1914, CXLVIII, 688; Lyon and Eiman, *Ibid.*, 885; Stephens, *Arch. Diag.*, 1915, VIII, 126; Schlesinger, *Med. Record*, 1915, LXXXVII, 61; Bana, *Brit. Med. Jour.*, 1915, I, 587; Stitt and Clark, *U. S. Naval Med. Bull.*, 1915, VIII, 410; Clark, *Ibid.*, 411; Basila, *Presse Med.*, 1915, XXIII, 364; Heidingsfeld, *N. Y. Med. Jour.*, 1916, CIII, 673; Bogomolets, *Russk. Vrach.*, 1916, XV, 1180; Hirschfelder, *Jour. A. M. A.*, 1916, LXVI, 1386; Fildes and McIntosh, *Lancet*, 1916, II, 751; Kafka and Haas, *Med. Klin.*, 1916, XII, 1312; Wehrlein, *Jour. Infect. Dis.*, 1916, XIX, 806; Ottenberg, *Jour. Immunol.*, 1916, II, 30; Smith and MacNeal, *Ibid.*, 75; Wade, *Jour. Med. Res.*, 1916, XXXIV, 113; Sellards and Minot, *Ibid.*, 131; Bronfenbrenner, and Schlesinger, *Am. Jour. Syph.*, 1917, I, 406; Thompson, *Ibid.*, 555.

Kolmer's Standardized Method.¹

As most serologists have adopted individual methods of conducting this complement-fixation test, it is not surprising that variable results are not infrequently reported by different workers with the same serum, especially when one recalls the complex nature of the Wassermann reaction and the variable properties of its different biologic reagents. To prevent and overcome such contingencies, if for no other reasons, it is greatly to be desired that a standardized technic be elaborated, so that all workers in this field may obtain concordant results and be able to "check up" on the same specimen of serum. Kolmer has recently introduced such a "standardized" method, which offers much hope that the problem has been solved, although some phases of it may later be found to require modification to meet conditions at present little understood or undetermined. From personal experience with this test in the writer's laboratory, both before (through the courtesy of Dr. Kolmer) and after publication of the technic, the writer can recommend this method of Kolmer as giving quantitative results which are to be relied upon.

Equipment.

Glassware.—Good pipets are essential, certified ones being best. It is advisable to have a separate 1 c.c. pipet for each serum, those pipets graduated to the tips being preferred as long as the tips are not chipped; otherwise pipets graduated to within 2 cm. of the tips are to be used. Such pipets should be graduated in 0.05 or 0.01 c.c. 5 and 10 c.c. pipets, graduated into 0.5 c.c. are also required. The test-tubes should have rounded bottoms, no lips and measure 85 mm. in length with an internal diameter of 12 to 14 mm. It is important that the diameter be within these limits on account of the color scale. For mixing large volumes, as in the preparation of corpuscle suspension or dilutions of complement serum, volumetric flasks rather than

Craig, *Ibid.*, 802; Bartlett and O'Shansky, *Ibid.*, 776; Jour. Lab. and Clin. Med., 1917, III, 118; Williamson, *Ibid.*, 1917, II, 202, 266 and 268; van Saun, *Ibid.*, 1917, III, 59; Huddleson, Jour. Immunol., 1917, II, 147; Sherwood, Jour. Infect. Dis., 1917, XX, 185; Callary, Jour. Lab. and Clin. Med., 1918, IV, 140; Ruediger, Jour. Infect. Dis., 1918, XXIII, 533; Dennie and Smith, Am. Jour. Syph., 1918, II, 101; Kolmer, *Ibid.*, 739; Victors, *Ibid.*, 758; Larkin, Cornwall, and Levy, Jour. Lab. and Clin. Med., 1919, IV, 571; Jeanselme and Bloch, Bull. Med. Paris, 1919, XXXIII, 533; Thomas and Simon, Canadian Med. Jour., 1919, IX, 1016; Arnaud, C. R. soc. de biol., 1919, LXXXII, 310; Rubinstein and Radosavlievitch, *Ibid.*, 361; Kolmer, Jour. Immunol., 1919, IV, 403; Wang, Jour. Path. & Bact., 1919, XXIII, 151; Ruediger, Ann. Med., 1920, I, 54; Breuer, Jour. Lab. & Clin. Med., 1920, V, 327; Kilduffe, *Ibid.*, VI, 98; Seelman, *Ibid.*, 144; Letulle, Presse Med., 1920, XXVIII, 588; Durupt, *Ibid.*, 636; Nobecourt and Bonnett, *Ibid.*, 745; Gradwohl, Chic. Med. Record, 1920, XLII, 413; Blaiyas, Jour. Lab. & Clin. Med., 1920, V, 244; Ruediger, Journal-Lancet, 1920, XI, 565; Thalhimier, Modern Med., 1920, II, 417; Lewis, Lancet, 1920, I, 11; Brooks, Jour. Med. Res., 1920, XLI, 399; Neukirch, Ztschr. f. Immunität., 1920, XXIX, 177; Breinl, *Ibid.*, 463; Kopaczewski, C. R. Acad. des Sc., 1920, CLXXI, 1170; Seelman, Am. Jour. Syph., 1920, IV, 157; Kolmer, *Ibid.*, 166; Henes, *Ibid.*, 685; Sanford, *Ibid.*, 697; Forssman, Biochem. Ztschr., 1921, CXXI, 180; Wadsworth and Maltener, Jour. Exp. Med., 1921, XXXIII, 110; Peyre, Presse Med., 1921, XXIX, 56; Kahn, Jour. Exp. Med., 1921, XXXIV, 217; Kahn and Olin, Jour. Inf. Dis., 1921, XXIX, 630; Kahn, Johnson and Boyd, *Ibid.*, 639; Kahn and Lyon, *Ibid.*, 651; Kahn, Jour. Lab. & Clin. Med., 1921, VI, 153 and 218; McIntyre, North and McIntyre, *Ibid.*, 233; Hadjopoulos, *Ibid.*, 624; Mahr, *Ibid.*, 1921, VII, 1; McNeil, *Ibid.*, 109; Hinton, Am. Jour. Syph., 1921, V, 81; Craig, *Ibid.*, 392; Larkin, *Ibid.*, 476; Wang, Lancet, 1922, I, 274; Browning, Dunlop and Kenaway, Jour. Path. & Bact., 1922, XXV, 36.

¹ Am. Jour. Syph., 1922, VI, 82.

the usual graduated cylinders should be used because of greater accuracy. The glass-stoppered graduated cylinders (50 to 100 c.c.) are more convenient for measuring intermediate amounts. For measuring any amount of fluid under 50 c.c., it is better to use an accurate 10 c.c. pipet. It is imperative that all glassware, including new material, should be chemically clean, that is, free of all traces of acids and alkalis and preferably sterile.

Test Tube Racks.—Each test requires six test tubes. Galvanized wire racks, carrying 12 rows of 6 tubes each, have been found very serviceable.

Water-bath.—A simple and inexpensive water-bath for heating sera and conducting the secondary incubation should be at hand.

Refrigerator.—Any refrigerator maintaining a temperature of between 6 and 8°C. suffices for the primary incubation.

Saline Solution.—This is an 0.85 per cent. solution of sodium chlorid in water prepared as follows: Keep C. P. sodium chlorid in a tightly stoppered bottle; if sufficient moisture has collected to render the salt lumpy, dry a portion in the oven before weighing. Weigh out 8.5 grams and dissolve in 1000 c.c. of freshly distilled water in a chemically clean and dry flask; filter through a good paper and sterilize by heating in an Arnold for an hour. Do not sterilize in an autoclave in order to avoid possible concentration. Before using in the Wassermann reaction, it is well to test the tonicity of the solution by adding a drop of washed blood cells to 5 c.c. of the solution in a test tube; if there are no immediate signs of lysis or none after gentle mixing and standing aside for $\frac{1}{2}$ hour, the solution is acceptable.

Corpuscles (Indicator Antigen).

This is a 2 per cent. suspension of freshly collected and washed sheep corpuscles; dose 0.5 c.c. Corpuscle suspension should be fairly uniform and may be prepared as follows: In washing defibrinated blood, one volume of blood is placed in a centrifuge tube, two volumes of saline solution added and gently mixed (citrate blood collected in the proportion of 1 part blood to 4 parts citrate solution is used without further dilution). Each tube is accurately counterbalanced in the centrifuge and whirled until all corpuscles have been thrown down. The supernatant fluid is carefully removed down to the corpuscles with a capillary pipet and at least 3 to 5 volumes of saline solution added; by capping the tube and inverting, all the corpuscles are thoroughly but gently stirred and mixed in the saline solution, and the tube again centrifuged (second washing). The supernatant fluid is again removed, replaced with saline solution, the corpuscles thoroughly mixed, and again centrifuged in accurately graduated tubes for twice as long as was found necessary to throw down the corpuscles in the second washing. If the supernatant fluid is discolored with hemoglobin, the cells should be washed again until the fluid is practically colorless. With the last washing the centrifuge is stopped slowly so as not to disturb the corpuscles, and the volume of the corpuscles read in the tube before removing the supernatant fluid. The latter fluid is now removed and a suspension of proper strength prepared by washing the cells from the centrifuge tube into a proper flask with 49 times as much saline solution as corpuscles. As the corpuscular suspension tends

to settle rapidly, the suspension should be gently and thoroughly shaken by hand before being used and during the time of use. When not in use, the suspension should be kept in a refrigerator.

Hemolysin.

This is antisheep hemolysin (amboceptor) diluted with saline and titrated daily with 0.3 c.c. of 1:30 guinea pig complement and 0.5 c.c. of 2 per cent sheep corpuscles.

Complement.

A 1:30 dilution of the mixed sera of several healthy guinea pigs. This complement must be (1) highly sensitive to fixation by antibody and antigen, (2) possess a high degree of hemolytic activity for the erythrocytes of the indicator antigen, and (3) be free or largely so of agglutinins and hemolysins for the cells of the indicator antigen. The complement may be preserved by adding to each cubic centimeter of serum 0.3 gram of chemically pure sodium chlorid and allowing it to dissolve; keep in a dark glass bottle at or near the freezing point. When it is to be used, dilute 1 c.c. with 29 c.c. of distilled water, which gives a 1:30 dilution in 1 per cent. saline. Such preserved sera are good at least 3 weeks. When using fresh complement serum, dilute 0.2 c.c. with 5.8 c.c. of saline solution (1:30), this is sufficient material for the hemolysin and complement titrations. The balance of serum should be placed in the ice-box and diluted later for the main tests.

TITRATION OF HEMOLYSIN AND COMPLEMENT

Titration of Hemolysin. -1. Arrange a series of 10 test tubes and place 0.5 c.c. of varying dilutions of hemolysin in each tube, respectively.

2. Ordinarily a range of dilutions from 1:5000 to 1:18000 is sufficient but depending upon the hemolytic activity of the complement and resistance of the corpuscles higher or lower dilutions may be required. A 1:100 dilution preserved with 0.2 per cent. phenol against bacterial contamination may be prepared as follows and kept in a refrigerator for several weeks, from which the higher dilutions are prepared as needed:

Equal parts hemolytic serum and glycerin.....	2.0 c.c.
Physiological saline.....	94.0 c.c.
5 per cent. phenol in saline or water.....	4.0 c.c.

3. The dilutions are prepared as follows in a separate set of large test tubes:

0.2 c.c. of 1:100	+ 1.8 c.c. saline = 1:1000
0.2 c.c. of 1:100	+ 3.8 c.c. saline = 1:2000
0.2 c.c. of 1:100	+ 5.8 c.c. saline = 1:3000
0.2 c.c. of 1:100	+ 7.8 c.c. saline = 1:4000
0.2 c.c. of 1:100	+ 9.8 c.c. saline = 1:5000
0.5 c.c. of 1:3000	+ 0.5 c.c. saline = 1:6000
0.5 c.c. of 1:4000	+ 0.5 c.c. saline = 1:8000
0.5 c.c. of 1:5000	+ 0.5 c.c. saline = 1:10000
0.5 c.c. of 1:6000	+ 0.5 c.c. saline = 1:12000
0.5 c.c. of 1:8000	+ 0.5 c.c. saline = 1:16000
Mix contents of each tube very thoroughly.	

4. Use 0.5 c.c. of each dilution in regulation test tubes and to each add 0.3 c.c. of 1:30 dilution of the same complement and 0.5 c.c. of a 2 per cent.

suspension of the same corpuscles as used in the complement-fixation tests; add 1.7 c.c. saline to each tube to make the total volume in each 3 c.c.

5. Mix the contents of each tube and place in the water-bath at 38°C. for one hour; *the unit is the highest dilution of hemolysin showing just complete hemolysis. Two units are employed in the titration of complement and antigen and in the complement-fixation tests.*

Table I shows the ensemble and results of a titration.

TABLE I.—TITRATION OF HEMOLYSIN

Tube	Hemolysin	Complement 1:30	Corpuscles 2 per cent.	Saline	After water bath incubation
1	0.5 c.c. 1: 5,000	0.3 c.c.	0.5 c.c.	1.7 c.c.	Complete hemolysis
2	0.5 c.c. 1: 6,000	0.3 c.c.	0.5 c.c.	1.7 c.c.	Complete hemolysis
3	0.5 c.c. 1: 7,000	0.3 c.c.	0.5 c.c.	1.7 c.c.	Complete hemolysis
4	0.5 c.c. 1: 8,000	0.3 c.c.	0.5 c.c.	1.7 c.c.	Complete hemolysis
5	0.5 c.c. 1: 9,000	0.3 c.c.	0.5 c.c.	1.7 c.c.	Complete hemolysis
6	0.5 c.c. 1: 10,000	0.3 c.c.	0.5 c.c.	1.7 c.c.	Complete hemolysis; unit
7	0.5 c.c. 1: 12,000	0.3 c.c.	0.5 c.c.	1.7 c.c.	Marked hemolysis
8	0.5 c.c. 1: 14,000	0.3 c.c.	0.5 c.c.	1.7 c.c.	Marked hemolysis
9	0.5 c.c. 1: 16,000	0.3 c.c.	0.5 c.c.	1.7 c.c.	Slight hemolysis
10	0.5 c.c. 1: 18,000	0.3 c.c.	0.5 c.c.	1.7 c.c.	No hemolysis

In the above titration the unit was 0.5 c.c. of 1:10,000; two units were contained in 0.5 c.c. of 1:5000.

6. Sufficient hemolysin is now prepared for the complement titration and complement-fixation tests so that two units are contained in 0.5 c.c.

Titration of Complement.

1. Arrange 10 test tubes and place 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 c.c. of 1:30 complement in each respectively; the tenth tube serves as a corpuscle control for this and the hemolysin titration.

2. Into each of the first nine tubes place 10 units of antigen so diluted that this amount is contained in 0.5 c.c.

3. Place sufficient saline solution in each tube to make the total volume about 2 c.c.

4. Mix contents of each tube and place in a water bath at 38°C. for one hour.

5. Add 0.5 c.c. hemolysin (2 units) and 0.5 c.c. corpuscle suspension (2 per cent.) to each tube and re-incubate one hour.

6. Ordinarily the smallest amount of 1:30 complement giving complete hemolysis is taken as the unit *but experience has shown that with the method of primary incubation employed in the complement fixation tests namely, 15 to 18 hours at 6-8°C. this is insufficient; in this test the unit is taken as the amount of complement in the next higher tube.* For example, if hemolysis is just complete with 0.25 c.c. the unit is taken as 0.3 c.c. and double this amount used for the antigen titrations and complement-fixation tests. For convenience Kolmer designates this amount as *two full units of complement*.¹

¹ Experience has shown that in this test the reactions are unsatisfactory if less than 0.4 c.c. of 1:30 complement is employed; occasionally hyperactive sera yield a unit with 0.4 c.c. of 1:30 but when this occurs it is necessary to use 0.4 c.c. for the dose of complement.

Table II shows the ensemble, the results of a titration and the method of reading.

TABLE II.—TITRATION OF COMPLEMENT

Tube	Complement 1:30	Antigen (10 units)	Saline	for one hour	Hemolysin (2 units)	Corpuscles (2°)	After Water-bath incubation for one hour
1	0.1 c.c.	0.5 c.c.	1.4 c.c.	Water Bath 38° C.	0.5 c.c.	0.5 c.c.	No hemolysis
2	0.15 c.c.	0.5 c.c.	1.4 c.c.		0.5 c.c.	0.5 c.c.	Slight hemolysis
3	0.2 c.c.	0.5 c.c.	1.3 c.c.		0.5 c.c.	0.5 c.c.	Marked hemolysis
4	0.25 c.c.	0.5 c.c.	1.3 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis; the exact unit
5	0.3 c.c.	0.5 c.c.	1.2 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis; the full unit
6	0.35 c.c.	0.5 c.c.	1.2 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
7	0.4 c.c.	0.5 c.c.	1.1 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
8	0.45 c.c.	0.5 c.c.	1.1 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
9	0.5 c.c.	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
10	—	—	2.0 c.c.		0.5 c.c.	0.5 c.c.	No hemolysis

In practice the hemolysin titration may be placed in the water-bath at the same time as the complement titration; at the end of the first incubation of the complement titration the unit of hemolysin is available and two units added to all tubes of the complement titration, etc.

7. Each two full units of complement are diluted with sufficient saline solution to make 1 c.c. called the dose of complement; for example if 0.3 c.c. of 1:30 complement is the full unit the dose is 0.6 c.c.

A convenient scheme for diluting the dose of complement to 1 c.c. is as follows: divide 30 by the dose = the dilution to employ in dose of 1 c.c.
For example:

Exact unit = 0.25 c.c. of 1:30 dilution.

Full unit = 0.3 c.c. of 1:30 dilution.

Two full units = 0.6 c.c. of 1:30 dilution.

$$\frac{30}{0.6} = 1:50$$

If 75 doses of complement were to be provided (sufficient for testing 12 sera or spinal fluids) this would require 75 c.c. of 1:50 prepared by diluting 1.5 c.c. guinea pig serum with 75.5 c.c. of saline solution.

Titration of Antigen.

The antigen employed in the complement fixation test for syphilis introduces the most important single factor of variation; the adoption of a certain kind of antigen fulfilling certain requirements is the most important factor in relation to standardization of technic.

In Kolmer's opinion an antigen should be an alcoholic extract of a fresh tissue and preferably heart muscle re-enforced with 0.2 per cent. cholesterin; this amount of cholesterin "stabilizes" the extract and greatly increases antigenic activity with very slight or no increase of anticomplementary activity

and without increasing the chances for nonspecific positive reactions with heated normal human sera. A superior antigen in my experience is that described below.

Technic for Preparing the Antigen.

Drying of Tissue.—Fresh beef or human hearts are washed free of blood, dissected free of fat and large blood vessels and the muscle passed through a meat grinder three or four times. The minced tissue is then rapidly dried in a vacuum apparatus or equally well by spreading in *thin layers* on clean glass plates and drying by *rapid fanning*, preferably in a dust proof box, for eighteen to twenty-four hours, turning the layers after the first ten to twelve hours. The cakes of dried material are now broken up, placed in an incubator over night and ground into a fine powder, which is kept in tightly stoppered bottles of colored glass at room temperature. *Three or more hearts may be prepared at one time and a mixture of the powders used in preparing extracts. Rapid drying is essential to prevent decomposition which results in greatly increasing the hemolytic activity of the extracts.*

Extractions.—(a) Twenty-five grams of powdered muscle are extracted with about 100 c.c. of ether in a Soxhlet for eighteen hours; if this apparatus is not available the powder is extracted with 200 c.c. of ether in a tightly stoppered bottle at room temperature for five days, being shaken occasionally each day. The ether is carefully removed and saved for the time being in a tightly stoppered bottle.

(b) The powder is now dried by fanning for a few minutes or by spreading on a glass plate for several hours, placed in a bottle and extracted with 200 c.c. of 95 per cent. alcohol in an incubator for four days.

(c) The alcohol is carefully decanted, poured into a flat shallow dish and fanned dry. The residue is extracted with 30 to 50 c.c. of ether for the ether soluble portion; this ethereal extract is covered and allowed to stand for an hour or two for the heavy insoluble particles to settle out.

The ethereal extract is now mixed with the ether of primary extraction and the mixture is concentrated by fanning until reduced to about one quarter volume or about 25 to 30 c.c. Six volumes or about 150 c.c. of pure acetone are now added to the concentrated ether which throws down a whitish precipitate; the mixture is covered and placed aside for several hours or over night for the complete separation of the acetone insoluble portions. On the following day the supernatant acetone is decanted and the sticky residue of acetone insoluble lipoids removed and kept in acetone in a tightly stoppered wide mouthed bottle for future use.

(d) The muscle powder is now extracted for a second time with 100 c.c. of absolute acetone free ethyl alcohol in an incubator for six days guarding against evaporation and shaking the mixture once or twice a day and if possible for at least one day in a mechanical shaker; the extract is now filtered through fat-free paper, measured and alcohol added to 100 c.c.

Finishing the Antigen.—Two-tenths gram pure cholesterol (Kahlbaum's preferred) and all of the acetone insoluble lipoids previously prepared, are dissolved in 10 c.c. of pure ether and the cloudy brownish mixture slowly

added to the filtered alcoholic extract and well shaken. The extract is now placed in the incubator for a few hours or over night, being shaken occasionally and then in a refrigerator for a day or two; the light brownish precipitate is now removed by filtration through fat-free paper or by decanting the extract. The finished antigen is kept in a tightly stoppered brown glass bottle in a refrigerator. Any precipitate forming after this time is left undisturbed.¹

Whatever antigen is employed it should be used in a dose of 10 antigenic units and this amount should be at least 20 times less than the anticomplementary and hemolytic units.

Antigen should be carefully preserved and titrated at least once a month unless it shows evidence of losing in antigenic activity or acquiring increased anticomplementary activity. Antigen should be diluted by placing the required amount of physiological saline solution in a test tube or Erlenmeyer flask and adding the required amount of extract drop by drop or in amounts of 0.1 c.c. and shaking by rotating after each addition.

Hemolytic and Anticomplementary Titration.—In a series of ten test tubes prepare the following dilutions of antigen:

1.0 c.c. antigen to 3.0 c.c. saline = 1:4
0.5 c.c. antigen to 2.0 c.c. saline = 1:5
0.5 c.c. antigen to 2.5 c.c. saline = 1:6
1.0 c.c. antigen 1:4 to 1.0 c.c. saline = 1:8
1.0 c.c. antigen 1:5 to 1.0 c.c. saline = 1:10
1.0 c.c. antigen 1:6 to 1.0 c.c. saline = 1:12
1.0 c.c. antigen 1:8 to 1.0 c.c. saline = 1:16
1.0 c.c. antigen 1:10 to 1.0 c.c. saline = 1:20
1.0 c.c. antigen 1:12 to 1.0 c.c. saline = 1:24
1.0 c.c. antigen 1:16 to 1.0 c.c. saline = 1:32

Hemolytic Titration.—1. In a series of ten regulation test tubes place 0.5 c.c. of the above dilutions of antigen, respectively.

2. To each tube add 0.5 c.c. of a 1:10 dilution of normal human serum previously heated for fifteen minutes at 55°C. and 1.5 c.c. of saline solution.

3. Mix the contents of each tube and place in a refrigerator at 6–8°C. for 15 to 18 hours.

4. Add 0.5 c.c. of 2 per cent. corpuscles suspension to each tube; mix and place in a water-bath at 38°C. for one hour.

5. Allow tubes to stand several hours in a refrigerator and read the results; *the smallest amount of antigen just beginning to produce hemolysis is the hemolytic unit.*

Table III shows the ensemble, the results of a titration and the method of reading.

Anticomplementary Titration.—1. In the first ten tubes of a second series of twelve tubes place 0.5 c.c. of the above dilutions of antigen, respectively.

2. To each of the first eleven tubes add 0.5 c.c. of a 1:10 dilution of normal human serum previously heated at 55°C. for fifteen minutes.

3. Add 1 c.c. of diluted complement (carrying two full units) to each of the twelve tubes.

4. To the eleventh tube add 0.5 c.c. and to the twelfth tube 1 c.c. of saline solution.

¹ Am. Jour. Syph., 1922, VI, 74.

TABLE III.—HEMOLYTIC TITRATION OF ANTIGEN

Tube	Antigen 0.5 c.c.	Heated human serum ¹ 1:10	Saline		Corpus- cles (2 per cent.)	Water-bath 1 hour
				Refrigerator 6-8°C. for 15-18 hours		
1	1:4	0.5 c.c.	1.5 c.c.		0.5 c.c.	Marked hemolysis
2	1:5	0.5 c.c.	1.5 c.c.		0.5 c.c.	Slight hemolysis; unit
3	1:6	0.5 c.c.	1.5 c.c.		0.5 c.c.	No hemolysis
4	1:8	0.5 c.c.	1.5 c.c.		0.5 c.c.	No hemolysis
5	1:10	0.5 c.c.	1.5 c.c.		0.5 c.c.	No hemolysis
6	1:12	0.5 c.c.	1.5 c.c.		0.5 c.c.	No hemolysis
7	1:16	0.5 c.c.	1.5 c.c.		0.5 c.c.	No hemolysis
8	1:20	0.5 c.c.	1.5 c.c.		0.5 c.c.	No hemolysis
9	1:24	0.5 c.c.	1.5 c.c.		0.5 c.c.	No hemolysis
10	1:32	0.5 c.c.	1.5 c.c.		0.5 c.c.	No hemolysis

¹ May be omitted in which case 2 c.c. saline are added to each tube instead of 1.5 c.c.

5. Mix all tubes and place in a refrigerator at 6 to 8°C. for 15 to 18 hours.

6. Place tubes in a water-bath at 38°C. for 5 to 10 minutes (not longer) and then add 0.5 c.c. hemolysin (two units) and 0.5 c.c. of the 2 per cent. suspension of corpuscles to each tube; mix and place in a water-bath at 38°C. for 1 hour. Place the tubes in a refrigerator for a few hours and read the results.

7. The *anticomplementary unit* is the smallest amount of antigen producing some inhibition of hemolysis. The eleventh tube is the serum control; the twelfth tube is the hemolytic system control and both should show complete hemolysis.

Table IV shows the ensemble, the results of a titration and method of reading:

TABLE IV.—ANTICOMPLEMENTARY TITRATION OF ANTIGEN

Tube	Anti- gen	Heated human serum ¹ 1:10	Comple- ment (2 full units).		Hemo- lysin (2 units)	Corpus- cles (2 per cent.)	Water-bath 1 hour
				Refrigerator 6-8°C. for 15-18 hours, followed by water-bath five to ten minutes.			
1	1:4	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Slight inhibition of hemolysis
2	1:5	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Slight inhibition of hemolysis (unit)
3	1:6	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
4	1:8	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
5	1:10	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
6	1:12	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
7	1:16	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
8	1:20	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
9	1:24	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
10	1:32	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
11	0.5 saline	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis
12	1.0 saline	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.	Complete hemolysis

¹ May be omitted and 0.5 c.c. saline added instead.

Antigenic Titrations.—1. In a series of ten test tubes prepare the following dilutions of antigen starting with the remainder of the 1:10 dilution prepared above for the hemolytic and anticomplementary titrations:

0.1 c.c. antigen 1:10	to 2.9 c.c. saline = 1:300
0.1 c.c. antigen 1:10	to 3.9 c.c. saline = 1:400
0.1 c.c. antigen 1:10	to 4.9 c.c. saline = 1:500
1.0 c.c. antigen 1:300	to 1.0 c.c. saline = 1:600
1.0 c.c. antigen 1:400	to 1.0 c.c. saline = 1:800
1.0 c.c. antigen 1:500	to 1.0 c.c. saline = 1:1000
1.0 c.c. antigen 1:600	to 1.0 c.c. saline = 1:1200
1.0 c.c. antigen 1:800	to 1.0 c.c. saline = 1:1600
1.0 c.c. antigen 1:1000	to 1.0 c.c. saline = 1:2000
1.0 c.c. antigen 1:1200	to 1.0 c.c. saline = 1:2400

2. Arrange a series of twelve regulation test tubes and place 0.5 c.c. of the above dilutions of antigen into the first ten tubes respectively.

3. In each of the first eleven tubes place 0.5 c.c. of a 1:10 dilution of a *mixture of equal parts of four or more freshly collected syphilitic and Wassermann positive sera previously heated at 55°C. for at least fifteen minutes.*

4. In each tube place 1 c.c. of diluted complement (carrying two full units).

5. To the eleventh tube add 0.5 c.c. and to the twelfth tube 1 c.c. of saline solution.

6. Mix contents of all tubes and place in a refrigerator at 6–8°C. for 15 to 18 hours.

7. Place tubes in a water-bath at 38°C. for 5 to 10 minutes (not longer) and then add 0.5 c.c. hemolysin (two units) and 0.5 c.c. of the 2 per cent.

TABLE V.—ANTIGENIC TITRATION OF ANTIGEN

Tube	Anti- gen 0.5 c.c.	Heated syphi- litic sera 1:10	Comple- ment (2 full units)	Hemoly- sin (2 units)	Corpus- cles (2 per cent.)	Water-bath 1 hour
1	1:300	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	Complete inhibition of hemolysis
2	1:400	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	Complete inhibition of hemolysis
3	1:500	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	Complete inhibition of hemolysis
4	1:600	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	Complete inhibition of hemolysis
5	1:800	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	Complete inhibition of hemolysis
6	1:1000	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	Complete inhibition of hemolysis
7	1:1200	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	Complete inhibition of hemolysis; unit
8	1:1600	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	Marked inhibition of hemolysis
9	1:2000	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	Marked inhibition of hemolysis
10	1:2400	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	Slight inhibition of hemolysis
11	0.5 saline	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	Complete hemolysis
12	1.0 saline	—	0.5 c.c.	0.5 c.c.	0.5 c.c.	Complete hemolysis

Refrigerator at 5–8°C. for 15–18 hours followed by water-bath five to ten minutes.

corpuscle suspension to all tubes; mix and place in a water bath at 38°C. for one hour. Place tubes in a refrigerator for a few hours and read the results.

8. The *antigenic unit* is the highest dilution of antigen giving complete inhibition of hemolysis. The eleventh tube is the serum control and the twelfth tube the hemolytic system control; both should show complete hemolysis.

Table V shows the ensemble, the results of a titration and method of reading.

9. Ten antigenic units are used in conducting the complement-fixation tests. For example, if the unit is 0.5 c.c. of 1:1000 dilution as shown in Table V, the dose of ten units would be contained in 0.5 c.c. of 1:100 dilution.

Technic of the Quantitative Complement-fixation Test.

1. Sera should be properly prepared and heated in a water-bath at 55°C. for fifteen minutes; spinal fluids are used unheated. The tests should be set up in the following order: serum first, followed by antigen, and lastly by complement.

2. In the test as originally published the tubes for the test were charged with diluted serum in the following dilutions; 0.1, 0.02, 0.004, 0.002, 0.001. As experience has shown the drop from 0.02 of serum to 0.004 is too great. For this reason Kolmer has changed these dilutions and recommends their use instead of the earlier strengths (personal communication). The dilutions to be used are prepared as follows: Place 1.2 c.c. of saline solution in tube 1 of each set; place 0.5 c.c. of saline in tubes 2, 3, and 5 of each set and 2 c.c. of saline in tube 4 of each set. Place 0.3 c.c. of serum in tube 1 and mix by drawing up in pipet and expelling at least 3 times. Transfer 0.5 c.c. of this mixture to tubes 2 and 6. Mix No. 2 thoroughly and transfer 0.5 c.c. to No. 3. Mix No. 3 and transfer 0.5 c.c. to No. 4. Mix No. 4 and transfer 0.5 c.c. to No. 5, discarding 1.5 c.c. of the mixture in No. 4. Mix No. 5 and discard 0.5 c.c. This leaves 0.5 c.c. of fluid in each of the six tubes of a set carrying the following dilutions: 0.1, 0.05, 0.025, 0.005, 0.0025, and 0.1 c.c. (serum control).

3. For each *spinal fluid* arrange six regulation tubes and place 0.5 c.c. saline in Nos. 2, 3, 4, 5 and 6.

Into the first, second and sixth tubes place 0.5 c.c. spinal fluid; mix No. 2 and transfer 0.5 c.c. to No. 3; mix No. 3 and transfer 0.5 c.c. to tube No. 5. (e) Mix No. 5 and discard 0.5 c.c.

Each tube now contains 0.5 c.c. carrying 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.5 c.c. (control).

4. Into the first five tubes of each set add 0.5 c.c. antigen dilution (carrying ten antigenic units).

5. After an interval of five to thirty minutes add 1.0 c.c. of complement to each tube (carrying two full units).

6. Include the following controls: (a) *antigen control* tube carrying 0.5 c.c. of the diluted antigen, 1 c.c. of the diluted complement and 0.5 c.c. of saline solution; (b) *hemolytic system control* carrying 1 c.c. of the diluted

complement and 1 c.c. of saline solution; (c) *corpuscle control* carrying 2 c.c. of saline solution; (d) *positive* and *negative* serum controls should be included using syphilitic and normal sera, respectively, set up in the various amounts as described above.

7. Start the preparation of a reading scale as follows:

a. Heat 6 c.c. of the diluted complement in a water-bath at 55°C. for 15 minutes.

b. Prepare a solution of hemoglobin by dissolving 2 c.c. of the 2 per cent. corpuscle suspension in 4 c.c. of plain water.

c. Arrange a series of five regulation test tubes numbered 1 to 5 and place in each: 0.5 c.c. of the diluted antigen and 1 c.c. of the heated diluted complement.

d. Add hemoglobin solution to the first four tubes as follows: 1.5, 1.13, 0.75 and 0.38 c.c.

e. Add 0.24 c.c. physiologic saline solution to No. 2; 0.5 c.c. to No. 3; 0.74 c.c. to No. 4 and 1 c.c. to No. 5.

Corpuscles are added the following day.

8. Mix the contents of all tubes gently but thoroughly and place in a refrigerator at 6 to 8° C. for 15 to 18 hours.

9. Warm the tubes in a water-bath at 38°C. for *five to fifteen minutes but no longer*¹ and add 0.5 c.c. hemolysin (carrying two full units) to all tubes except the corpuscle control; thoroughly mix the 2 per cent. corpuscle suspension (which has been carried over in a refrigerator from the previous day) and add 0.5 c.c. to all tubes except those of the reading scale.

10. Add corpuscle suspension to the tubes of the reading scale as follows: 0.13 c.c. to No. 2; 0.25 c.c. to No. 3; 0.38 c.c. to No. 4 and 0.5 c.c. to No. 5.

11. Mix the contents of all tubes gently but thoroughly and place in a water-bath at 38°C. for 1 hour (the water must reach above the level of the contents of the tubes).

12. Place all tubes in a refrigerator for 1 to 3 hours to permit the partial settling of non-hemolyzed corpuscles; the degree of inhibition hemolysis is then read off and recorded for each tube with the aid of the reading scale as —, + (1), ++ (2), +++ (3) or ++++ (4). All serum controls, antigen and hemolytic controls should show complete hemolysis.

13. Tube No. 1 of the color scale shows a — reaction; tube No. 2 shows a +; tube No. 3 shows a ++; tube No. 4 shows a +++ and tube No. 5 shows a ++++ reaction.

Experience has shown that the reactions may be interpreted as follows:

Very strongly positive if there is partial or complete fixation of complement in the first four or all five of the fixation series.²

¹ This preliminary warming may be omitted; if more than 15 minutes are used some non-specific reactions may occur.

² Occasionally a serum will show less fixation of complement in the first tube carrying 0.1 c.c. serum than the second tube carrying 0.02 c.c. as ++, ++++, +++, and + and — (control) reaction (recorded as 2, 4, 3, 1—). It may be assumed that this is due to the presence of natural antisherp hemolysin but the phenomenon may occur with hemolysin free serum due probably, to the presence of other serum constituents in this relatively large amount of serum interfering with the fixation of complement by antigen and syphilis antibody. This does not seem to occur with spinal fluids.

Strongly positive if there is partial or complete fixation of complement in the first three tubes.

Moderately positive if there is partial or complete fixation of complement in the first two tubes.

Weakly positive if there is partial or complete fixation of complement in only the first tube.

Negative when all tubes show complete hemolysis.

The following results of tests with a syphilitic serum and spinal fluid shows the method of recording and reporting.

Quantitative Reaction.—Strongly Positive (4421 -).

Serum 0.1	c.c. = + + + +
Serum 0.02	c.c. = + + + +
Serum 0.004	c.c. = + +
Serum 0.002	c.c. = +
Serum 0.001	c.c. = -
Serum 0.1 (control)	c.c. = -

Quantitative Reaction.—Very Strongly Positive (44442).

Spinal Fluid 0.5	c.c. = + + + +
Spinal Fluid 0.25	c.c. = + + + +
Spinal Fluid 0.125	c.c. = + + + +
Spinal Fluid 0.0625	c.c. = + + + +
Spinal Fluid 0.03125	c.c. = + +
Spinal Fluid 0.5 (control)	c.c. = -

TABLE VI.—THE QUANTITATIVE COMPLEMENT-FIXATION, TEST, FOR SYPHILIS

Tube	Patient's serum ¹ in 0.5 c.c.		Antigen 10 units	Complement (2 full units)	Primary incubation at 6-8° C. for 15-18 hours followed by water- bath for 5 to 10 minutes.	Hemoly- sin (2 units)	Corpus- cles (2 per cent.)
1	0.1	c.c.	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.
2	0.05	c.c.	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.
3	0.025	c.c.	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.
4	0.005	c.c.	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.
5	0.0025	c.c.	0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.
6	0.1	c.c.	-	1.0 c.c.		0.5 c.c.	0.5 c.c.
	(control)						
7	Antigen		0.5 c.c.	1.0 c.c.		0.5 c.c.	0.5 c.c.
	Control						
8	Hemolytic		-	1.0 c.c.		0.5 c.c.	0.5 c.c.
	Control						
9	Corpuscle		-	2.5 c.c.		-	0.5 c.c.
	Control			saline			

¹ Spinal fluid doses: 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.5 c.c. (control).

Diagnostic Value of the Wassermann Test.

As stated above, the Wassermann reaction, or any of its modifications, is not a specific immunity reaction. While the antibody is, presumably, syphilitic in origin, the antigen is a physiologic lipid and not a product of the spirochete *Treponema pallidum*. In other words, the complement fixation test, as applied to the diagnosis of syphilis, is not the result of an interaction between homologous (specific) antigen and antibody. Nevertheless, we find that the reaction does have a certain specificity from the diagnostic, if not from the

biologic, standpoint. There is no question but that the technic of the Wassermann Test should be standardized, so as to permit of more certainty in comparison of results of different workers. This is especially to be desired for those laboratories which conduct examinations for physicians in various parts of the country. It is, today, almost impossible to arrive at conclusions, when reports from different laboratories are at variance, owing to the variability of the technic employed. Attempts have been made to standardize this technic and suggestions have been offered by several workers, but up to the present time no accepted solution of this important phase of the work has been attained.¹ (See page 739.)

As a general rule it may be stated, at the outset, that a positive Wassermann test, all proper precautions and controls being made, indicates syphilis in practically all cases. It is true that certain nonspecific conditions, especially leprosy and, occasionally, scarlet fever, give positive reactions, but the large majority of cases of other diseases, reported as giving positive results, are those in which syphilis has not been absolutely excluded. In such cases the syphilitic infection may be latent instead of the active cause of the trouble at the time of making the test, yet it must be reckoned with in deciding as to the value of this test from the diagnostic point of view.²

On the other hand, it is to be distinctly understood that a negative Wassermann test does not exclude syphilis, a fact that must be taken into consideration when the question of marriage is involved. Such cases must be judged largely by the clinical manifestations and history rather than upon the negative character of this test. Varying percentages of positive results in known syphilitic and parasymphilitic conditions have been reported by various workers. Some of the more enthusiastic ones claim that a negative Wassermann reaction means that the case is not syphilitic. This view, however, must be regarded as untenable in the face of the large numbers of negative results in the literature and in the daily experience of those working in this field.

¹ See Stillians, *Am. Jour. Syph.*, 1917, I, 767; Ottenberg, *Arch. Int. Med.*, 1917, XIX, 457; Emery, *Lancet*, 1918, II, 547; Kolmer, *Am. Jour. Syph.*, 1919, III, 1; Brown and Kolmer, *Ibid.*, 8; Kolmer and Brown, *Ibid.*, 170; Kolmer, Matsunami and Trist, *Ibid.*, 407 and 513; Kolmer and Flick, *Ibid.*, 1919, III, 541; Todd, *Southern, Med. Jour.*, 1919, XII, 667; Wolbarst, *N. Y. Med. Jour.*, 1920, CXL, 177; Solomon, *Jour. A. M. A.*, 1920, LXXIV, 788; Kolmer, Trist and Flick, *Am. Jour. Syph.*, 1920, IV, 111; Kolmer and Rule, *Ibid.*, 135; Kolmer, Matsunami and Rule, *Ibid.*, 278; Kolmer and Rule, *Ibid.*, 484; Kolmer, Matsunami and Rule, *Ibid.*, 518; Hinton, *Ibid.*, 598; Kolmer, Rule and Trist, *Ibid.*, 616, 641 and 675; Kolmer and Trist, *Ibid.*, 1921, V, 30; Kolmer, Rule and Yagle, *Ibid.*, 44; Kolmer, Matsunami and Trist, *Ibid.*, 63; Kolmer, *Ibid.*, 290; Ray, *Ibid.*, 320; Kolmer, *Jour. Am. Med. Assoc.*, 1921, LXXVII, 776; *Am. Jour. Syph.*, 1921, V, 439, 451, 641 and 628; *Ibid.*, 1922, VI, 74, 82, 289 and 319.

² See Kolmer, *Jour. Exper. Med.*, 1911, XIV, 235; Field (*Jour. Am. Med. Assn.*, 1912, LVIII, 1681) has called attention to the occurrence of positive Wassermann tests in cases of lead poisoning. Hilgermann, *Deutsch. med. Wchnschr.*, 1912, XXXVIII, 118; Gibson, *Australasian Med. Gaz.*, 1913, XXXIII, 300. For other diseases showing a Wassermann see Jakobovics, *Jahrb. f. Kinderhke.*, 1914, XXIX, 215; Fletcher, *Lancet*, 1914, I, 1677; Butler, *U. S. Naval Med. Bull.*, 1915, IX, 51; Hesse, *Wien. klin. Wchnschr.*, 1915, XXVIII, 62; Sutherland and Mitra, *Indian Jour. Med. Research*, 1915, II, 984; Verdozzi and Urbani, *Policlinico*, 1915, XXII, Med. Sec., 529; Prins, *Ned. Tijdschr. v. Geneesk.*, 1916, II, 1562; Sonntag, *Deutsche Med. Wchnschr.*, 1916, XLII, 1577; Thomson and Mills, *Lancet*, 1919, CXCVI, 782; Iyengar, *Indian Jour. Med. Res.*, 1919, VII, 407; *Ibid.*, VIII, 136; Duhot and Crampon, *Bull. de la Soc. Méd. des Hôp.*, 1921, XLV, 587; Bauer, *Münch. med. Wchnschr.*, 1921, LXVIII, 1251.

The following table, compiled from Boas¹ and Noguchi² shows the reported cases of syphilis with the percentages of positive results.³

Considerable discussion has arisen in the literature regarding the relative merits of the Wassermann and Noguchi systems. As a general rule it is found, by one running these two systems in parallel, that the positive results

¹ Die Wassermannsche Reaktion., Berlin, 1922.

² Serum Diagnosis of Syphilis, Philadelphia, 1912.

³ See Lucas, Am. Jour. Dis. Child., 1912, III, 259; Churchill, Ibid., 363; Kaplan, Med. Record, 1912, LXXXI, 1132; Bates, Arch. Int. Med., 1912, X, 470; De Buys, Am. Jour. Dis. Child., 1913, V, 65; Milne, Am. Jour. Med. Sc., 1913, CXLV, 197; Wolbarst, New York Med. Jour., 1913, XCIV, 378; Grindon, Davis, Greiner and Weiss, Interstate Med. Jour., 1913, XX, 221; Sarateanu and Velican, Monatsschr. f. Geburtsh. u. Gynäk., 1913, XXXVII, 89; Balcarek, Med. Klin., 1913, IX, 1552; Eliasberg, Deutsch. Ztschr. f. Chir., 1913, CXXIV, 113; Blackfan, Nicholson and White, Am. Jour. Dis. Child., 1913, VI, 162; Holt, Ibid., 166; Richards, Jour. Am. Med. Assn., 1913, LX, 1139; Kaplan, New York Med. Jour., 1913, XCVIII, 308; McIlroy, Watson and McIlroy, Brit. Med. Jour., 1913, II, 1002; Heidingsfeld, Jour. Am. Med. Jour., 1913, LXI, 1598; Mensi, Gazz. d. osp., 1913, XXXIV, 1032; Post, Boston, Med. and Surg. Jour., 1913, CLXIX, 777; Kaplan, Jour. Am. Med. Assn., 1913, LXI, 2214; Emery, Lancet, 1914, I, 223; Philippson, Policlinico, 1914, XXI, 20; Russo, Riv. osped., 1914, IV, 173; Descomps, Grèce, Méd., 1914, XV, 37; Lesser and Klages, Deutsch. med. Wchnschr., 1914, XL, 1309; White, Therap. Gaz., 1914, XXXVIII, 307; MacKinney, Ann. of Surg., 1914, LX, 309; Bahr, Illinois Med. Jour., 1914, XXV, 245; Corbus, Ibid., 1914, XXVI, 112; Craig, Jour. Am. Med. Assn., 1914, LXII, 1232; Thomas and Ivy, Am. Jour. Med. Sc., 1914, CXLVIII, 55; Southard, Boston Med. and Surg. Jour., 1914, CLXX, 947; Stone, Med. Record, 1914, LXXXVI, 545; Fordyce, New York Med. Jour., 1914, C, 597; Craig, Am. Jour. Med. Sc., 1915, CLXIX, 41; Krauss, Biochem. Ztschr., 1915, LXVIII, 48; Pontano, Policlinico, 1915, XXII, 41, 77 and 113; von Gonzenbach, Cor.-Bl. f. Schweiz. Aerzte, 1915, XLV, 161 and 225; Wolbarst, Interstate Med. Jour., 1915, XXII, 109; Gradwohl, Southern Med. Jour., 1915, VIII, 477; Robertson and Klauder, Jour. Am. Med. Assn., 1915, LXIV, 199; Keyes, Ibid., 804; Vedder and Hough, Ibid., 972; Weisenburg, Ibid., 975; Haberman, Ibid., 1141; Heimann, Ibid., 1463; Wile and Stokes, Ibid., 1465; Glomset, Ibid., 1915, LXV, 682; Uhle and MacKinney, Ibid., 863; Moore, Ibid., 1980; Whitney, Ibid., 1986; Walker and Haller, Ibid., 1916, LXVI, 488; Haller, Ibid., 882; Kolmer, Ibid., 1435; Falls and Moore, Ibid., LXVII, 574; Gougerot, Paris Med., 1916, VI, 181; Lowrey, Am. Jour. Ins., 1916, LXXII, 601; Buhman, Surg. Gyn. and Obs., 1916, XXIII, 284; McNeil, Southern Med. Jour., 1916, IX, 202; Courtney and Shropshire, Ibid., 205; Gordon, Arch. Pediat., 1916, XXXIII, 273; Uhle and Mackinney, Boston Med. and Surg. Jour., 1916, CLXXIV, 55; Med. Times, 1916, XLIV, 301; Simons, Jones and Goddard, Interstate Med. Jour., 1916, XIII, 654; Graves, Jour. Immunol., 1916, II, 53; Moursund, Texas State Jour. Med., 1916, XII, 435; Judd, Am. Jour. Med. Sc., 1916, CLI, 836; Warthin and Wilson, Ibid., CLII, 157. Snow and Cooper, Ibid., 185; Fordyce, Ibid., 469; DeBuys and Langford, Am. Jour. Dis. Child., 1916, XII, 387; Ladd, N. Y. Med. Jour., 1916, CIV, 952; Craig, Mil. Surg., 1916, XXXVIII, 286; Am. Jour. Syph., 1917, I, 192; Peterson, Ibid., 211; Knapp, Ibid., 772; Ball, Jour. A. M. A., 1917, LXVIII, 262; Slemmons, Am. Jour. Med. Sc., 1917, CLIII, 212; Schmidt, Chicago Med. Rec., 1917, XXXIX, 185; Kaplan, N. Y. Med. Jour., 1917, CV, 728; Gehrmann, Am. Jour. Pub. Health, 1917, VII, 964; Wassermann, Berl. klin. Wchnschr., 1917, LIV, 105; Freudenberg, Ibid., 303; Heller, Ibid., 300; Scheel, Ugesk. f. Laeger, 1917, LXXIX, 43; Slack, Castleman and Bailey, Boston Med. and Surg. Jour., 1917, CLXXXVII, 180; Mayer, Berl. klin. Wchnschr., 1918, LV, 86; Freudenberg, Ibid., 620; Lambert, Olmstead, and Stuart, Proc. N. Y. Path. Soc., 1918, XVIII, 61; Special Report No. 21, Medical Research Committee, National Health Insurance, London, 1918; Day and McNitt, Am. Jour. Syph., 1919, III, 595; Ravant, Presse Méd., 1919, XXVII, 120; Goodman, Surg. Gyn. & Obs., 1920, XXX, 368; Durupt, Presse Méd., 1920, XXVIII, 636; Nicolas and Gaté, Ann. de dermatol. et de syphiligraph, 1920 (VI S), I, 3; Wagner, Ztschr. f. Immunität., 1920, XXX, 26; Williams, Bull. Johns Hopk. Hosp., 1920, XXXI, 335; Scott and Pearson, Am. Jour. Syph., 1920, IV, 201; Sargent, Ibid., 286; Oettinger, Ibid., 207; Royster, Ibid., 1921, V, 131; Williams, Ibid., 284; Jackson and Pike, Jour. Am. Med. Assoc., 1921, LXXVI, 360; Nagel, Calif. State Jour. Med., 1921, XIX, 103; Simon, Arch. Dermatol. & Syph., 1921, III, 630; Kahn, Jour. Lab. & Clin. Med., 1921, VI, 579; Dock, Jour. Missouri State Med. Assoc., 1921, XVIII, 339; Menze, Münch. med. Wchnschr., 1921, LXVIII, 1200; Fordyce and Rosen, Jour. Am. Med. Assoc., 1921, LXXVII, 1606; Mills, Edinb. Med. Jour., 1922, XXVIII, 19; Kilduffe, Arch. Dermatol. and Syph., 1922, V, 207; Strickler, Jour. Am. Med. Assoc., 1922, LXXVIII, 962.

Condition	Number of cases	Positive results
Primary syphilis.....	1,060	59.0 per cent.
Secondary syphilis.....	3,526	90.0 per cent.
Tertiary syphilis.....	1,212	84.1 per cent.
Early latent syphilis.....	1,233	51.0 per cent.
Late latent syphilis.....	1,520	39.0 per cent.
Congenital syphilis.....	125	94.5 per cent.
Cerebrospinal syphilis.....	64	47.6 per cent.
General paralysis.....	498	88.1 per cent.
Tabes dorsalis.....	360	70.0 per cent.

are somewhat higher with the Noguchi system. The following table, taken from Noguchi, gives the figures for the cases reported, in which the two tests were made upon the same serum.

Condition	Number of cases	Wassermann positive	Noguchi positive
Primary syphilis.....	208	88 per cent.	94 per cent.
Secondary syphilis.....	669	92 per cent.	98 per cent.
Tertiary syphilis.....	455	74 per cent.	83 per cent.
Latent syphilis.....	305	54 per cent.	68 per cent.
Congenital syphilis.....	79	98 per cent.	98 per cent.
Cerebrospinal syphilis.....	55	73 per cent.	80 per cent.

Effect of Treatment on the Reaction.

Under the influence of a vigorous course of mercurials, a positive Wassermann test may be made to disappear. This must, however, not be interpreted as meaning that the syphilitic infection has been cured. It is almost daily observed by those doing large numbers of these tests that a patient, having the test made while under active treatment with mercury, will frequently give a negative test; while the same patient may show a positive reaction if the mercury be withdrawn for two or three weeks prior to the test. On the other hand, we occasionally find cases in which the treatment has resulted in a positive Wassermann, prior tests having been negative. These facts must be borne in mind by those who may attempt to establish a diagnosis without full knowledge of the conditions under which the test is being made. Mercurial treatment may render the infection latent, so that a negative reaction may obtain until later active manifestations appear and become associated with a positive test. This variability has been shown by Craig,¹ who reports 52 cases treated, with 32 positives and 20 negatives after treatment ranging from two weeks to twelve years. Patients should not have had any mercurial treatment for at least two to three weeks prior to the test if any reliance at all is to be placed upon it. Patients well treated give negative reactions, while those receiving inadequate treatment, no matter for how long a period, show positive results. The reaction is, unquestionably, affected by mercurial treatment, but some cases persist in giving a positive reaction in

¹ Jour. Exper. Med., 1910, XII, 726; Arch. Int. Med., 1911, VIII, 395. See Nelson and Anderson, Jour. A. M. A., 1915, LXV, 1905; Goodman, Arch. Dermatol. & Syph., 1920, II, 103. Craig and Nichols (Jour. Am. Med. Assn., 1911, LVII, 474) and Craig (Ibid., 1913, LX, 565) call attention to the influence of alcohol in converting a positive test into a negative one within 24 hours.

spite of treatment. The reaction may return shortly after treatment, while it is not, by any means, settled that the disappearance of a reaction justifies one in stopping treatment. It is definitely established that a number of cases of syphilis, which show positive Wassermann tests, continue to show this positive phase in spite of the most vigorous treatment. This is the so-called "Wassermann-fast" type. Just what factors are accountable for this state have not been determined. McNeil states that "When the number of complement-binding units in the serum of a patient remains stationary under intensive treatment or is increased, it would seem safe to conclude that the spirochetes have become immune to the drug or drugs that are being administered; the patient, in other words, is "Wassermann-fast" and will remain so until the treatment is changed to a more effective one." It is evident, therefore, that, in some cases at least, a prolonged treatment with any single drug or combination of drugs is liable to result eventually in the establishment of this condition.¹

After treatment with "606" (salvarsan, dioxy-dimino-arseno benzol), the results are variable. In promptly cured cases, a positive reaction may disappear within two weeks or it may require four to five weeks to obtain a negative result. A positive reaction may persist for considerable time and then disappear, reappearing again after an uncertain period. It has been definitely established that, if a patient be syphilitic, a negative Wassermann can be converted into a positive one by an injection of salvarsan, owing to the fact that the spirochete are killed and their toxic action is temporarily increased, the bodies concerned in the Wassermann test being thereby increased. This is the so-called "Provocative Wassermann Reaction" and can be obtained in every stage of infection.² The blood for the test should be taken before the injection as a control and then 24 and 48 hours after the administration of $\frac{1}{2}$ dose of the drug. Occasionally it is wise to take the blood one to two weeks after the injection also, in cases which are of doubtful nature. While it is the general experience of workers in this field that the serum of an individual with latent syphilis, giving a weakly positive or even a negative Wassermann reaction, may show a temporary positive "provocative" test, yet, it is also established that a positive Wassermann reaction in a syphilitic may be changed to a negative one by arsphenamine treatment, except in those cases which are "Wassermann-fast." Strickler, Munson, and Sidlick, however, have reported a study of a series of 24 non-syphilitic cases in which intravenous arsphenamine treatment caused the appearance of positive Wassermann reactions in 16 of the subjects, 14 of the 16 giving at least 2 or more positive results, one patient 9 and another 6. The importance of these findings, if substantiated, are great upon the value and interpretation of the test. As Kolmer points out, it is possible that some of these cases had latent syphilis and that arsphenamine brought out the "provocative" phase and that his own experiments on rabbits have shown him that arsphen-

¹See Stokes and Busman, *Am. Jour. Med. Sc.*, 1920, CLX, 658; McNeil, *Jour. Am. Med. Assoc.*, 1921, LXXVII, 1970.

²See Stokes and O'Leary, *Am. Jour. Syph.*, 1917, I, 629; O'Leary, *Arch. Dermatol. and Syph.*, 1920, II, 348.

amine does not produce positive Wassermann tests in animals which showed no tendency to give such a test prior to the treatment. He is, therefore, "unconvinced that arsphenamine produces such changes in non-syphilitics" as the above workers have indicated. Kilduffe has recently shown, in confirmation of Kolmer, that injection of arsphenamine into rabbits resulted in uniformly negative Wassermann tests. It would seem, therefore, that it is doubtful that non-syphilitics will respond to arsphenamine with positive tests unless there is a latent or unsuspected specific infection present.¹

In deciding as to the proper criterion of a cure in any case a negative test following a positive one has little value. To establish a basis for the assertion of a cure, one should insist that the following points be met. One year without treatment, without any suspicious signs, with several negative Wassermann reactions and no positive ones, and with a negative provocative Wassermann reaction and luetin test at the end of the year.²

β. Diseases Other Than Syphilis.³

The complement-fixation test is, theoretically, applicable to any infectious disease. Acute infections, however, do not show positive results until sufficient time has elapsed to permit of absorption of enough toxin to cause a systemic response to invasion, as shown by the presence of specific antibodies in the serum tested. Sub-acute and chronic cases offer the best chances for positive results. Practically, however, the tests have not been equally satisfactory in all types of infection or invariably reliable in the same type.

The principle of the test is the same as described above no matter what the condition under investigation. The hemolytic system may be either sheep or human, the reagents used being as mentioned with the exception of the antigen employed. These antigens are polyvalent in character, being prepared from several different strains of the suspected organism. As different strains of the same organism often differ markedly one from another, the possibility exists of the serum of an infected patient fixing complement only in the presence of closely allied strains. Hence, the more strains of the organism in any given antigen the more certain will be the results of the test.

The antigens are prepared as follows: Cultivate as many different strains as possible of the organism on appropriate media, such as agar slants or blood-agar slants, and wash the surface of each slant with 2 or 3 c.c. of a 0.9 per cent. solution of sodium chlorid, scraping the organisms from the media with a platinum loop. Pour off the suspension into a sterile tube and shake thoroughly. Kill the organisms by heating the tube in a water-bath at 60°C. for

¹ See Pearce and Kolmer, *Jour. Inf. Dis.*, 1916, XVIII, 32; Strickler, Munson and Sidlick, *Jour. Am. Med. Assoc.*, 1920, LXXV, 1488; Kolmer, *Ibid.*, 1796; Katsaines, *Ibid.*, 1921, LXXVI, 195; Kilduffe, *Ibid.*, 1490.

² See Nichols, *Jour. Am. Med. Assn.*, 1912, LVIII, 603; also, Craig and Nichols, *Studies of Syphilis*, Bull. 3, War Dept., Office of Sur. Gen., Washington, 1913; Pusey, *Am. Jour. Med. Sc.*, 1913, CXLVI, 497. Corbus (*New York Med. Jour.*, 1914, C, 472) reports a case of second infection, the Wassermann having been negative for one and one-half years. See King, *Jour. A. M. A.*, 1916, LXVII, 1669; Trimble and Rothwell, *Ibid.*, 1984, LeComte, *Am. Jour. Syph.*, 1919, III, 106.

³ See Ludke in *Handbook of Kraus and Levaditi*, *Ergänzungsband 1*, 1911, 518; *Ztschr. f. klin. Med.*, 1911, LXXII, 545.

one-half hour. Standardize the number of bacteria so that 1 c.c. of the suspension contains 1,000 million bacteria (see last chapter). The amount of antigen to be used in the tests must then be determined by titration just as described for the Wassermann test.

Gonorrhea.

In systemic gonorrheal infections the complement-fixation test is becoming of considerable importance. The technic is carried out as given above. The antigen should consist of at least twelve strains of gonococci. In the writer's laboratory the human hemolytic system is employed exclusively and the results have been constant and satisfactory. Others prefer the sheep system. The same precautions must be followed here as in the Wassermann test, every point in the technic being carefully controlled.

A positive reaction indicates a focus of active gonorrheal infection in the system; while a negative result does not exclude this condition, owing to the variability in the antigens mentioned above. The influence of treatment upon this test is not at all clear, but it must be remembered that all cases treated with vaccines will show strong positive reactions. Just how long after such treatment is discontinued one may expect to obtain a positive result is still unsettled, so that the absolute determination of a cure must rest largely on the clinical findings. This is an important point, especially where marriage is contemplated, as the possibility of infecting another must be kept in mind. Non-infectivity cannot be predicated on a single negative fixation test.¹

Attempts to use this method, as a diagnostic procedure, have been made in typhoid fever, tuberculosis,² meningococcic infections, cholera, pertussis,

¹ See Schwartz and McNeil, *Am. Jour. Med. Sc.*, 1911, CXLI, 693; Swinburne, *Arch. Diagnosis*, 1911, IV, 227; Schwartz, *Am. Jour. Med. Sc.*, 1912, CXLIV, 369; Schwartz and McNeil, *Ibid.*, 815; Lenartowicz, *Tygodnik lekarski*, 1912, VII, 368; *Dermatol. Wchnschr.*, 1912, LV, 1179; Lespinasse and Wolff, *Illinois Med. Jour.*, 1913, XXIII, 26; Smith, *Am. Jour. Dis. Child.*, 1913, V, 313; Finkelstein and Gerschun, *Berl. klin. Wchnschr.*, 1913, L, 1817; McNeill, *Arch. Pediat.*, 1913, XXX, 657; McDonagh and Kline, *Jour. Path. and Bacteriol.*, 1913, XVII, 559; Thomas and Ivy, *Arch. Int. Med.*, 1914, XIII, 143; Smith, *Am. Jour. Dis. Child.*, 1914, VII, 230; Williams, *Interstate Med. Jour.*, 1914, XXI, 1198; Kolmer and Brown, *Jour. Infect. Dis.*, 1914, XV, 6; Thomas, Ivy and Birdsall, *Surg., Gyn. and Obs.*, 1914, XIX, 390; *Arch. Int. Med.*, 1915, XV, 265; Irons and Nicoll, *Jour. Infect. Dis.*, 1915, XVI, 303; Sormani, *Nederl. Tijdschr. v. Geneesk.*, 1915, I, 1077; Uhle and MacKinney, *New York Med. Jour.*, 1915, CII, 737; Irons and Nicoll, *Trans. Chic. Path. Soc.*, 1915, IX, 305; Asch and Adler, *Munch. Med. Wchnschr.*, 1916, LXIII, 73; Warden and Schmidt, *Jour. Lab. and Clin. Med.*, 1916, I, 333; Magner, *Lancet*, 1920, II, 123; Smith and Wilson, *Jour. Immunol.*, 1920, V, 499.

² In tuberculosis the results of this test have been rather variable. Some reports are extremely enthusiastic, while others are of a doubtful nature. It would seem that the results with the antigen of Besredka (an autoclaved filtered culture of tubercle bacilli grown on a liquid medium composed of alkaline broth to which is added egg white and egg yolk) are the most reliable. However, even with this antigen, Bronfenbrenner reports as high as 20 per cent. of positive results with syphilitic cases, while Craig, on the other hand, shows that only one per cent. of syphilitics show positive results in his hands, when this antigen is applied. Of course, this may mean that many syphilitics are, also, infected with tuberculosis. Cook, in his work, calls attention to the fact that the bacterial antigens, as used in this test, are specific for the acid fast group of bacterial infections but that they are not capable of sufficient differentiation for diagnostic purposes. He believes that the test is of some value in calling attention to unrecognized tuberculosis, but does not always indicate a clinically active process. At present, complement fixation in tuberculosis can not be placed on the same level as in syphilis, either from the diagnostic or prognostic point of view. See Besredka, *Ztschr. f. Immunitätsforsch.*, 1914, XXI, 77; Bronfenbrenner, *Ibid.*, 1914, XXIII, 2 and 221; *Arch. Int. Med.*, 1914, XIV, 786; Stinson, *Bull. 101, Hyg. Lab.*, U. S. P. H. S., 1915, Craig, *Am. Jour. Med. Sc.*, 1915, CL, 781; Cooper, *Jour. Infect.*

small-pox, infection with pyogenic cocci and other infections but the results have not been conclusive or reliable. Likewise it has been employed in the diagnosis of infection with various animal parasites, such as in echinococcus disease; helminthiasis in general and trichinosis. Here, also, the results are still too uncertain to warrant the routine use of this test.¹

D. Abderhalden's Sero-diagnosis.²

1. Of Pregnancy.

Abderhalden has recently introduced a biologic test for pregnancy, which, he assumes, depends upon the presence of certain ferments found in the serum of pregnant animals. This test appears to be fairly certain in its results and should prove of some value to the gynecologist and obstetrician.

It will be recalled that the injection of foreign protein into the system results in the production of certain well-established biologic properties in the serum of the animal so injected. Among these properties one finds the formation of precipitins, agglutinins, anaphylactogens, etc. Abderhalden has shown that the parenteral injection (subcutaneous, intraperitoneal or intravenous) of foreign protein or carbohydrate brings about the appearance in the blood serum of proteolytic or amylolytic ferments, as an indication of the effort of the system to protect itself against the possible toxic effects of this non-hydrolyzed material. Such protective ferments are, he believes, specific and are never found in the serum of normal (non-injected) animals. The

Dis., 1916, XIX, 313; Bronfenbrenner, Kahn, Rockman, and Kahn, Arch. Int. Med., 1916, XVII, 492; Miller, Jour. Lab. and Clin. Med., 1916, I, 816; Miller, Jour. A. M. A., 1916, LXVII, 1519; Craig, Ibid., 1917, LXVIII, 773; Burns, Slack, Castleman, and Bailey, Ibid., 1386; McCaskey, Am. Jour. Med. Sc., 1917, CLIV, 648; Woods, Bushnell, and Maddux, Jour. Immunol., 1917, II, 301; Bronfenbrenner, Jour. Lab. and Clin. Med., 1917, III, 50; Blumberg, Ibid., 397; Brown and Petroff, Am. Rev. Tuberc., 1918, II, 525; Lange Ibid., 541; Stivelman, Ibid., 546; Wilson, Jour. Immunol., 1918, III, 345; von Wedel, Ibid., 351; Small, Ibid., 413; Stoll and Neuman, Jour. A. M. A., 1919, LXXII, 1043; Lewis, Am. Rev. Tuberc., 1919, III, 129; Young and Givler, Ibid., 476; Cooke, Jour. Infect. Dis., 1919, XXV, 493; Walthard, Cor. Bl. f. Schweiz. Aerzte, 1919, LXIX, 1577; Barnes and Bernton, Boston Med. and Surg. Jour., 1919, CLXXX, 38; Pritchard and Roderick, Jour. A. M. A., 1919, LXXIII, 1879; Moursund, Jour. Infect. Dis., 1920, XXVI, 85; Young and Givler, Am. Rev. Tuberc., 1919, III, 476; Rogers, Jour. Inf. Dis., 1920, XXVII, 101; von Wedel, Jour. Immunol., 1920, V, 159; Rénon, Médecine, 1920, I, 406; Hekman, Nederl. Tijdschr., 1920, I, 1612; Ott, Münch. med. Wchnschr., 1920, LXVII, 1130; Watkins and Bonyton, Jour. Am. Med. Assoc., 1920, LXXV, 933; Stivelman, Jour. Lab. & Clin. Med., 1920, V, 453; Upham and Blaivas, Ibid., 784; Punch, Lancet, 1920, II, 647; 1921, II, 497; Punch and Gosse, Brit. Med. Jour., 1922, I, 509; Kilduffe, Jour. Lab. & Clin. Med., 1922, VII, 427; Sellers and Ramsbottom, Jour. Path. and Bact., 1922, XXV, 247.

¹ See Kolmer, Arch. Int. Med., 1912, IX, 220; Hastings, Jour. Am. Med. Assn., 1913, LX, 1208; Besredka and Manoukhine, Ann. de l'Inst. Pasteur, 1914, XXVIII, 569; Compt. rend. Soc. de Biol., 1914, LXXVI, 197; Hastings, Jour. Infect. Dis., 1914, XX, 52 and 72; Garbat, Am. Jour. Med. Sc., 1914, CXLVIII, 84; Friedlander and Wagner, Am. Jour. Dis. Child., 1914, VIII, 134; Olmstead and Luttinger, Arch. Int. Med., 1915, XVI, 67; Winholt, Jour. Infect. Dis., 1915, XVI, 389; Howell, Ibid., 456; Nakajo and Asakura, Ibid., 1915, XVII, 388 and 400; Kolmer and Strickler, Jour. Am. Med. Assn., 1915, LXIV, 800; Olmstead and Povitsky, Jour. Med. Res., 1915, XXXIII, 379; Hirschfelder, Jour. A. M. A., 1915, LXV, 2073; Tribondeau and Fichet, Bull. de l'Acad. Med., 1916, LXXVI, 256; Kolmer and Trist, Jour. Infect. Dis., 1916, XVIII, 20 and 64; Kolmer, Trist and Heist, Ibid., 274 and 88; Kolmer and Pearce, Ibid., 32; Kolmer, Ibid., 46; Dick and Dick, Ibid., 1916, XIX, 175 and 638; Kolmer, Jour. Immunol., 1916, I, 51 and 59.

² Abderhalden, Abwehrfermente des tierischen Organismus, Berlin, 1914. See, also, Vaughan, Jour. Am. Med. Assn., 1914, LXIII, 365; Taylor and Hulton, Jour. Biol. Chem., 1915, XXII, 59; Adami, Jour. Canadian Med. Assn., 1915, V, 569. Pregl and de Crinis (Fermentf., 1917, II, 58) have devised a micro Abderhalden test.

blood will be seen, therefore, to have acquired definite digestive properties apart from that resident to a more or less extent in the leucocytes. A further step, taken by Abderhalden and his pupils, has been the demonstration that substances, which are native to the system but foreign to the blood, may arise from physiologic or pathologic changes within the system itself and, after absorption into the blood, produce quite as definite a response as if the material were introduced from without.

Schmorl and Veit have shown that syncytial cells and portions of the chorionic villi may become detached from the placenta and enter the maternal circulation, while other placental products (of an unknown nature) presumably also are either absorbed or washed into the blood current. As these substances are, in part at least, protein in nature, we should have, if the theory holds, a production of protective proteolytic ferments in the maternal blood, which are capable of digesting placental protein and are more or less specific. If, therefore, the serum of a suspected animal be treated with placental protein, hydrolytic cleavage must occur with the formation of products capable of more or less easy detection, provided the serum contains such ferments. These are the principles which form the basis of the test as originated by Abderhalden.

Since the introduction of this test an enormous amount of work has appeared dealing with its various phases. The earlier publications seemed to confirm the finding of Abderhalden that specific ferments were present in certain conditions, capable of digesting only so-called homologous protein. Many of the studies were adverse, the results being attributed by Abderhalden to faulty technic. During recent years numerous researches have been made, which seem to strike a somewhat discordant note. At the present time the status of this question may be summed up as follows: In every fresh serum, male or female, normal or abnormal, ferments are present in the blood which, under certain conditions, are capable of causing hydrolytic cleavage of protein. Unquestionably, these proteolytic ferments are found in increased amounts in cases of pregnancy, carcinoma and other conditions to which the Abderhalden test has been applied. These ferments are, however, not to be regarded as specific, in the sense that they digest only homologous protein added as a substrat, as it is very questionable whether they act at all on the tissue used in the Abderhalden test. Owing to the presence in the blood of normal antiproteolytic substances, these normal (and hence not defensive ferments) ferments cannot exert their activity. If, however, this so-called "antitrypsin" be removed from the blood (1) by extraction with chloroform or ether (Delezenne and Pozerski), (2) by inactivation by addition of 0.2 per cent. acetic acid to the serum (Opie), or (3) by adsorption with an organic substrat or substances such as kaolin, starch or agar (Plaut, Kjaergaard and others), changes occur in the degree of dispersion of the colloidal system and the normal proteolytic ferments become free to act. Sera treated in any of the above ways will undergo self-digestion and liberate dialyzable products capable of detection by the Abderhalden or other methods. The substrat (placental or other tissue) acts exactly in this way. Further investigations

have shown that this reaction is similar in many respects to that of combination of antigen and antibody as previously discussed, thus bringing the Abderhalden reaction into the realm of other immunity tests. According to Stephan, Hauptmann and, more recently, to Bronfenbrenner, the Abderhalden test is possible only when complement is present. If complement is missing, as when the serum is heated, any fresh serum will activate the test. From the later studies of Bronfenbrenner it seems probable that the reaction occurs in two stages: (1) the substrat (antigen) combines with the antibody, thus becoming sensitized; (2) this sensitized tissue then adsorbs the antitrypsin, leaving the normal proteolytic ferments of the blood free to digest the protein of the serum. In other words, the Abderhalden test does not depend upon the presence of specific ferments which digest only homologous tissue, but upon the presence of specific (probably) antibodies, which unite with the substrat and, in so doing, adsorb antitryptic bodies and render the normal proteolytic ferments active. In this sense the Abderhalden test is to be regarded as specific, as it is certain that positive reactions in suspected cases are far more numerous, under the most carefully controlled conditions, than are the negative findings.¹ However, the specificity is far removed from that claimed by Abderhalden,² and, hence, the diagnostic value of the test must be regarded as unproven.

¹ In recent years there has arisen a decided tendency, based upon an accumulation of experimental evidence, to ascribe various phenomena of immunity to variations in the colloidal dispersion of the serum proteins and lipoids. Bronfenbrenner (*Jour. Lab. and Clin. Med.*, 1916, I, 573) asserts in regard to the Abderhalden test that "the element of specificity lies, not in the ferment itself, but in the mechanism of its activation." The combination of specific serum with its corresponding antigen is followed by a radical change in the degree of dispersion of serum colloids. See, also, Jobling and Petersen, *Jour. A. M. A.*, 1916, LXVI, 1753.

² For discussions of the various points raised in this newer investigation see Gammeltoft, *Ugeskr. f. Laeger*, 1913, LXXV, 1247; Heilner and Petri, *Münch. med. Wchnschr.*, 1913, LX, 1530; Oeller and Stephan, *Ibid.*, 1914, LXI, 12, 75 and 425; Plaut, *Ibid.*, 238; de Waele, *Ibid.*, 364; Flatow, *Ibid.*, 468, 608, 1168 and 1500; Stephan, *Ibid.*, 801; Hauptmann, *Ibid.*, 1167; von Domarus and Barsieck, *Ibid.*, 1553; Freund and Brahm, *Ibid.*, 1664; Lindig, *Ibid.*, 1668; Beumer, *Ibid.*, 1999; Kjaergaard, *Ztschr. f. Immunitäts f. Orig.*, 1914, XXII, 31; de Waele, *Ibid.*, 170; Herzfeld, *Biochem. Ztschr.*, 1914, LXIV, 103; *Ibid.*, 1915, LXVIII, 402; Wilhelm and Szandicz, *Ibid.*, 1914, LXV, 219; Benech, *Compt. rend. Soc. de biol.*, 1914, LXXVI, 361; Bettencourt and Menezes, *Ibid.*, 1914, LXXVII, 162; Michaelis and Lagermarck, *Deutsch. med. Wchnschr.*, 1914, XL, 316; Fränkel, *Ibid.*, 589; Bisgaard and Korsbjerg, *Ibid.*, 1367; Mosbacher and Port, *Ibid.*, 1410; Peiper, *Ibid.*, 1467; Oeller and Stephan, *Ibid.*, 1557; Friedmann and Schönfeld, *Berl. klin. Wchnschr.*, 1914, LI, 348; Lange, *Ibid.*, 785; Csepai, *Wien. klin. Wchnschr.*, 1914, XXVII, 804; Franz, *Arch. f. Gynäk.*, 1914, CII, 579; Heinemann, *Monatsschr. f. Geburtsh. u. Gynäk.*, 1914, XXXIX, 768; Adachi, *Ztschr. f. Geburtsh. u. Gynäk.*, 1914, LXXVI, 516; Jobling and Petersen, *Jour. Exper. Med.*, 1914, XIX, 480; *Ibid.*, 1914, XX, 37; Bronfenbrenner, *Ibid.*, 1915, XXI, 221 and 480; Jobling, Eggstein and Petersen, *Ibid.*, 239; Jobling, Petersen and Eggstein, *Ibid.*, 1915, XXII, 120 and 141; Echols, *Jour. Am. Med. Assn.*, 1914, LXIII, 370; Eggstein, *Ibid.*, 735; Falls, *Ibid.*, 1172; *Ibid.*, 1915, LXIV, 1898; *Ibid.*, 1915, LXV, 524; Bronfenbrenner, Schlesinger and Mitchell, *Ibid.*, 1915, LXV, 1268; Bronfenbrenner, *Jour. Lab. and Clin. Med.*, 1915, I, 79; Jobling and Petersen, *Bull. Johns Hopk. Hosp.*, 1915, XXVI, 356; Jobling, Petersen and Eggstein, *Jour. Exper. Med.*, 1915, XXII, 568, 590, 597 and 603; Kabanow, *Fermentforsch.*, 1915, I, 206; Falls, *Trans. Chic. Path. Soc.*, 1915, IX, 303; Herzfeld, *Deutsche Med. Wchnschr.*, 1915, XLI, 1151; Van Slyke, Vinograd-Villchur, and Losee, *Jour. Biol. Chem.*, 1915, XXIII, 377; Hulton, *Ibid.*, 1916, XXV, 163 and 227; Lampert, Katz, King, Kline, Kulasavicz, Jeffries and Kutzenberger, *Ill. Med. Jour.*, 1916, XXX, 22; Oppler, *Biochem. Ztschr.*, 1916, LXXV, 211; Fujimoto, *Jour. Immunol.*, 1918, III, 51; Robertson and Hanson, *Ibid.*, 131; Linossier, *C. R. soc. biol. Paris*, 1918, LXXXI, 422; Yamakawa, *Jour. Exper. Med.*, 1918, XXVII, 689 and 711.

Two distinct methods of detecting the presence of these antibodies have been devised. The first, the optic method, is capable of very wide application to the diagnosis of different conditions and should prove extremely useful in solving many problems of great clinical importance. It requires, however, considerable skill and technical ability as well as rather expensive apparatus. The second, the dialyzation method, is much simpler both in technic and in necessary equipment. Both of these methods require the most assiduous attention to the various details given, if any dependence is to be placed upon the results of the tests.

1. The Optic Method.

The basis of this test is as follows: A solution of placental peptone in physiologic salt solution has a definite power of rotating the plane of polarized light. Likewise, the serum, both suspected and normal, has a similar action. The degree of rotation, however, of either remains permanent for some time at 37°C. If a solution of peptone and normal serum be mixed and the degree of rotation of this mixture determined, no appreciable change will be observed between the initial and final polarimetric readings. If, however, a solution of placental peptone, whose polarizing action is known, be treated with a serum containing the specific ferments above mentioned, digestion of the peptone occurs with the formation of products showing rotatory powers sufficient to change the initial rotation of the mixture to quite an extent. These changes may be observed at different intervals and interpreted as described later.

Preparation of Placental Peptone.

The fresh placenta is made blood-free by cutting it into small pieces and placing these under running water for about 15 minutes.¹ Dry the pieces between folds of filter paper and place them in about five times their weight of 70 per cent. sulphuric acid. Allow the mixture to stand for three days at room temperature and shake the container frequently. At the end of this time, place the container in ice-water and dilute the contents with 10 volumes of distilled water, stirring constantly. Remove the sulphuric acid by adding approximately the calculated amount of finely powdered barium hydrate and complete the precipitation with a known solution of this salt, stirring the mixture constantly. When the reaction of the mixture becomes neutral to litmus paper, filter off the barium sulphate. If the filtrate be turbid, refiltration is necessary until a perfectly clear filtrate is obtained. The separation of the barium sulphate is much facilitated by the use of the large centrifuge, if such be at hand. Wash the precipitate with a large amount of cold water and combine the filtrate and washings. Test the mixture for both barium and sulphuric acid. If either be present, it must be removed. Now evaporate this barium- and sulphuric acid-free solution to dryness on the water-bath, under reduced pressure at a temperature not exceeding 40 or 50°C. It is wise to test the evaporated material at several intervals for the presence of either barium or sulphuric acid, as these sometimes appear on concentrating the mixture. It is important that these be removed, as their presence will

¹ For discussions of the chemical composition of the placenta see Harada, *Acta Scholæ, Med. Univ. Kioto*, 1916, I, 283 and 291; Fenger, *Jour. Biol. Chem.*, 1917, XXIX, 19.

result in further hydrolysis of the peptone and, in consequence, will lessen the value of the final product. A thick yellow syrup or a foamy mass remains after this evaporation. The product may be used in this form, but it is preferable to purify it, if reliable results are to be invariable.

This yellowish residue is dissolved in methyl alcohol with the aid of heat and the hot solution is poured into absolute ethyl alcohol. The peptone is thrown down as a yellow powder, which is soluble in water to a clear yellowish solution of weakly acid or amphoteric reaction. This powder is not hygroscopic. A further purification is still advisable. Dissolve the above yellow powder in water up to a 5 per cent. solution and add 10 per cent. solution of phosphotungstic acid as long as a precipitate forms. Filter and wash several times with water. Rub up this precipitate in a mortar with some water and twice its weight of barium hydrate. Filter again and remove the excess of barium from the filtrate with sulphuric acid. Filter off the barium sulphate and evaporate the filtrate to dryness under reduced pressure at 40 to 50°, as outlined above. This product is snow white and is permanent.

It is absolutely essential for the successful application of the optic test that the placental peptone be as pure as possible. The same product is not always obtained by the above method, as the hydrolysis may proceed further than the peptone stage. Such products are unsuitable for the test. It is wise, therefore, to work as quickly and as carefully as possible with a large amount of placental substance, so that one may obtain an appreciable amount of placental peptone. If the product be found serviceable, it may be kept for years. A further point to be considered in the use of a prepared peptone is that the solution of this placental product must give absolutely no turbidity with the serum to be tested. Such a finding is not infrequent, owing to the probable presence of precipitins in the product. Such a peptone cannot be used.

A further important property of the prepared peptone must be its power of rotating the plane of polarized light. The degree must not be too small or the product will prove of little value. It will be seen, therefore, that the preparation of a serviceable and proper placental peptone is a matter of considerable difficulty and is paramount to the successful performance of the test.

One may preserve the peptone, prepared as above, either in the solid state or in the form of a solution. The advantage of a solution is that one has on hand a large amount of material, which will give good comparative results, as the solution is permanent. Abderhalden formerly used solutions of 0.5 to 2.5 per cent. strength. He now advises a 10 per cent. solution of the placental peptone in physiologic salt solution. This solution must be absolutely clear and colorless. If not, filter through thick paper or a Berkefeld. Preserve this clear solution by overlaying its surface with toluol. When required for the test, the solution is withdrawn by a pipet dipping below the toluol. If care be taken to keep a layer of toluol over the solution, the stock material will be permanent for a long period. Should this solution become turbid at any time, the material should be thrown away and a new stock solution prepared as

above. The optical activity of this stock solution must be tested before each test.¹

Obtaining the Serum.

The serum of the patient is obtained as described under the complement-fixation tests, withdrawing 10 to 15 c.c. of blood. Place the blood, as drawn, directly into a sterilized centrifuge tube, so that all cellular elements may be completely separated. For a successful test the serum must show no sign of the presence of cells. A further precaution to be taken is that no sign of hemolysis must be present in the serum. For this reason, the cells should be separated rapidly. It is wise to make the test on the same day on which the blood is taken, although, if the precautions above mentioned are observed, a delay of 24 to 48 hours does not materially affect the activity of the serum.

Technic.

Having prepared the 10 per cent. solution of placental peptone and having proven that it answers all the requirements above mentioned, place 1 c.c. of this clear solution (withdrawn by a pipet) in a small, clean, sterile test-tube. Add 2 c.c. of the clear suspected serum and shake the tube several times. Examine the mixture carefully for any turbidity or precipitation. If any be observed, the test cannot be carried out. Add sufficient physiologic salt solution to the mixture to fill the 1 decimeter polarimetric tube (see below). This mixture with salt solution is preferably made in this way, rather than to add the salt solution after the peptone solution and serum have been placed in the polarizing tube. Any turbidity may be much more easily detected. Pour the above mixture into the 1 decimeter tube, whose mantle has been filled with water at 37°C.

Carefully determine the initial rotatory power of the mixture, checking the readings several times and controlling them by subsequent ones after 5 or 10 minutes. No change should be observed in these readings. Place the tube and its contents in the incubator at 37°C. and repeat the readings every hour for a few periods and then continue every 6 or 8 hours. Do not extend the investigation over more than 48 hours. Record all readings and interpret them as given below.

Control tubes must be arranged as follows: (1) the peptone solution alone; (2) the suspected serum alone; (3) peptone solution plus normal serum; (4) peptone solution plus known positive serum; (5) peptone solution plus inactivated (heated to 60°C.) suspected serum. In all of these controls the same conditions must be maintained and the same length of polarizing tube must be used as in the test itself. If any turbidity occurs in any of the control mixtures or tubes, these must be disregarded in interpreting the test as turbid solutions give variable results with the polarimeter.

It goes without saying that this test requires the very best equipment possible. The cheap polariscopes are absolutely useless as they are not delicate enough to detect the fine variations given. The three-shadow instrument of Landolt-Lippich, made by Schmidt and Haensch, is especially to be

¹ The placental peptone may now be obtained on the market from the Höchst-Farwerke Co., New York.

recommended. The polarizing tubes used are, preferably, the 1 decimeter tubes which are furnished with a mantle which may be filled with water at any desired temperature. If different length tubes are used in any of the tests, a correction must be made in order that comparative figures may be obtained.

In performing this test, even to a greater degree than when the instrument is used in other work, much depends upon the ability of the worker to detect slight variations in the degree of the rotatory powers of the mixtures under investigation. The method is easy to learn, but the special sensibility toward such changes cannot be taught. Abderhalden cautions any one, who shows a working error of as much as 0.04° in his observations, against attempting to interpret the test.

In reporting the result of this test, Abderhalden employs the following method.

Deviations within 0.04°	Negative
Deviations between 0.05 and 0.1°	Positive (+)
Deviations between 0.11 and 0.2°	Positive (++)
Deviations over 0.2°	Positive. (+++)

2. The Dialyzation Method.

This method is much more simple than the optic method, both as regards technic and apparatus. It must not be thought, however, that any less care is necessary in carrying out the details of the test. In fact, erroneous results are, perhaps, more easily obtained by careless manipulation when this method is employed.

The basis of this method is the conversion of the colloidal non-dialyzable placental protein into dialyzable products through the activity of the ferments above mentioned. These products are, then, detected by simple color reactions in the dialysate.

Preparation of Placental Albumin.

Although Abderhalden does not regard the proteolytic ferments of the serum in pregnancy as absolutely specific for a given species of animal, he, nevertheless, insists that the protein preparations used in either of his methods be prepared from the placenta of the same species as that of the animal whose serum is to be tested.

As autolysis proceeds fairly rapidly in placental tissue, the albumin should be prepared from placentas which are as fresh as possible. Remove the external portions of the placenta, such as the membranes, and wipe away as much blood as possible. Cut the material into small pieces and wash for a short time in running water.¹ While this is being done, boil about 2 liters of water to which are added 2 drops of glacial acetic acid. Throw the washed bits of placenta into this boiling water and boil for 5 to 15 minutes. Pour the mixture upon a loose quick-acting filter and boil the pieces again with a second portion of acidulated water for 5 to 15 minutes. Pour off this water and test with the triketohydrinden hydrate reaction given below. If a positive reaction obtains, the placental tissue must be again boiled with

¹ It is essential that all visible blood be removed as its presence will introduce a large error.

acidulated water until a negative reaction occurs. The essential points in this process are rapid and complete coagulation of the placental albumin and the removal of all soluble dialyzable material which may react with the reagent mentioned above.¹

As soon as a negative result is obtained with the extractive water, pour the mixture into a wide-mouth flask, add some chloroform, overlay the fluid with toluol and stopper the flask; or place the material in several smaller glass jars and overlay with toluol. This placental albumin keeps almost indefinitely and may be removed from the containers as desired. It should be tested, from time to time, to show that it contains, in itself, nothing which may react with the reagents used in the later test.²

Obtaining the Serum.

The serum is obtained by venous puncture as previously described, the blood (about 10 c.c.) being drawn directly into a sterilized centrifuge tube. The cellular elements are separated as quickly as possible and the serum drawn off into a clean sterile tube. It is of especial importance in this test that the serum show no sign of hemolysis. As it has been shown that amino-acids are present in the blood during digestion and may, therefore, give a positive reaction with triketohydrinden hydrate, it is wise to take the blood in the morning before breakfast in all cases. If such be not done, a less amount of serum must be used in the later test to compensate for this possible error.

Selecting the Dialyzing Tubes.

It is evident that this part of the preparation for the test is of extreme importance. The dialyzing thimbles must be permeable for peptone but not for albumin. Unless these conditions obtain, the test is valueless. Not all of the thimbles, purchasable upon the market, are by any means available. Abderhalden advises the use of the diffusion shells No. 579A. of Schleicher and Schüll. Not all of these will answer the purpose.

It is necessary, therefore, that all the dialyzing thimbles used in the test should have been previously tested and known to answer the above requirements. As the thimbles are usually dry and hard when obtained, soak them in cold water for a few hours, place them in boiling water for a few seconds and keep them in water covered with toluol.

To test these thimbles for their permeability for albumin, proceed as follows. Remove the thimble from the water and place in it 5 c.c. of serum or of a solution of egg albumin. Add a few drops of toluol to prevent bacterial action. Place 20 c.c. of distilled water in the dialyzing vessel and overlay this with toluol. This dialyzing vessel should be quite narrow, the distance between the wall and the thimble (when in place) being about $\frac{1}{4}$ cm. These vessels are kept plugged with cotton and are sterilized before use. Now suspend the thimble with its albuminous contents in the dialyzing tube in such a way that the fluid outside is as high or, preferably, a little higher than that within the thimble. Plug the vessel with cotton to prevent contamination

¹ Care must be taken not to add an excess of acetic acid to the water as this may interfere with the ninhydrin test and thus give rise to an appreciable error.

² Abderhalden recommends that this placental albumin be tested with ninhydrin before being used in any test. This is a vital point.

and put the apparatus in the incubator at 37°C. for 18 to 24 hours. At the end of this time, test the dialysate (outside fluid) for albumin by the biuret or triketohydrinden hydrate reactions given below. Those thimbles giving negative results are retained to be tested for their permeability to peptone. The shells permitting the passage of albumin cannot be used in the test.

Select those thimbles showing impermeability to albumin and wash them thoroughly in water. Place in them 5 c.c. of a 1 to 1000 solution of Witte's or, preferably, peptone from silk (peptone La Roche) and add sufficient toluol to cover the solution. Dialyze as above against 20 c.c. of distilled water, placing the apparatus in the incubator for 18 hours at 37°C. Those thimbles which permit the passage of peptone, as shown by the triketohydrinden hydrate test, are then kept for use in these tests and the non-permeable ones are laid aside. The properly tested and selected thimbles are then preserved in water overlaid with toluol.

Technic.

Remove a few pieces of the coagulated placental albumin from the container, wash in distilled water and dry between filter paper.¹ Break this up into very small bits or grind up in a mortar. Weigh out three portions of $\frac{1}{2}$ gram each. Place $\frac{1}{2}$ gram in each of three tested dialyzing thimbles in such a way that none of the material touches or remains upon the top or outside of the shells. Carefully wash off the outside of the thimble by means of a stream of distilled water or hold it under running water. This is done to remove any possible adhering placental albumin, which would vitiate the test later made. Now add to tube No. 1, 1 to 1.5 c.c. of clear hemoglobin-free serum to be tested. This serum is withdrawn from its container by means of a sterile graduated pipet. Overlay the surface of the mixture in the thimble with toluol. This thimble is then placed in a sterile dialyzing tube, as described above, containing 20 c.c. of distilled water, which should stand slightly higher than the fluid in the thimble. Overlay the external fluid with toluol and plug the dialyzing vessel with cotton to prevent contamination. Place the apparatus in the incubator at 37°C. for 18 hours and then test the dialysate for peptone as outlined below.

Controls.

Charge thimble 2 with $\frac{1}{2}$ gram of placental albumin and 1 c.c. of serum of a known positive control. Overlay with toluol and arrange as above.

Charge thimble 3 with $\frac{1}{2}$ gram of placental albumin and 1 c.c. of a known negative serum or with the inactivated (heated to 60°C.) serum used in the test.

A further control should be run, using 1 c.c. of the serum alone without the addition of albumin, to prove that it does not contain any dialyzable substances which will give the later reactions.

The tests with all of these controls are carried out exactly as the test itself, every precaution being taken to prevent the introduction of errors. The tests for cleavage products of albumin are made with one of the following tests, the latter being in some respects preferable.

¹ Test the material before use with the triketohydrinden hydrate reaction. Absolutely no trace of a blue coloration should obtain.

The Biuret Test.

This was the test formerly employed by Abderhalden and has some advantages in that it does not react with certain dialyzable products not infrequently present in the serum of normal subjects. It requires considerable care and skill in manipulation as well as in interpretation. Doubtful results are very frequent unless every precaution be taken.

Remove about 10 c.c. of the dialysate by means of a pipet dipping below the toluol. Place this in a test-tube and add 5 c.c. of a 33 per cent. sodium hydrate solution. Mix by careful shaking and add very carefully, drop by drop, from a buret a very dilute (0.25 per cent.) solution of copper sulphate in such a way that a distinct contact ring is formed. If peptone is present, a violet-red to a pure red contact ring will be observed, sharply differentiated from the lower colorless and upper blue solutions. This is a positive reaction. A negative result is shown by the appearance of a distinctly blue ring. It is not the simplest matter to distinguish between the various shadings which occur, so that one must not make his decision without having had some experience in differentiating the colorations obtained with pure albumin and peptone solutions.

The Triketohydrinden Hydrate Reaction.

This reagent occurs in colorless crystals readily soluble in water. It may be obtained from the Höchst-Farbwerke (Lucius & Brüning) Company under the trade name of "ninhydrin." Its formula is $\text{C}_6\text{H}_4 \begin{array}{c} \diagup \text{CO} \diagdown \\ \diagdown \text{CO} \diagup \end{array} \text{C}(\text{OH})_2$.

It is of especial importance that every precaution be taken to prevent error when this reagent is used, as reactions may rise from the presence of substances which are not at all associated with the hydrolytic products of protein material. This reagent is not by any means specific, even for albumin, peptone or amino-acids, although it was formerly believed that it reacted only with substances containing an amino and a carboxyl group, the former especially in the α position. It has been shown that there is a larger number of compounds which are not in a chemical sense combinations with amino and carboxyl groups and which, nevertheless, give very characteristic reactions. Among these we find: amines; amino-aldehydes; urea derivatives; amino-sulphonic acids; ammonium derivatives of certain organic acids, dicarbonyl compounds and halogen-aldehydes; ammonium compounds of thiosulphuric, oxy-sulpharsenic and selenic acids; ammonium formate, ammonium thio-lactate, etc. Of special importance is the fact that a very small amount of basic products of putrefactive origin will give a decided reaction (hence the importance of using every means to prevent decomposition of the tissue).

Certain further precautions are essential. If any of the original placental albumin be left on the outside of the dialyzing thimble, a very obvious error will arise. If the serum used contains amino-acids, the amount must be determined by depth of color with the ninhydrin. One must avoid the presence of acid or ammoniacal fumes in the laboratory. Strong alkalis

cause, in themselves, a coloration with the reagent, while dilute alkalis may decolorize the solution. Acids prevent the appearance of the blue color and will destroy the color already formed, even in the presence of a large amount of reacting material. It must be insisted, therefore, that the fluid to be tested be absolutely neutral. Further, all vessels and pipets must be absolutely clean and the water used must be free from bacteria.¹

Remove 10 c.c. of the dialysate by means of a pipet dipping below the toluol and place this in a large test-tube. Add 0.2 c.c. of a 1 per cent. aqueous solution of triketohydrinden hydrate. Heat rapidly to the boiling point and keep the mixture boiling for one minute. If the reaction be negative, the solution remains colorless or becomes, at most, light yellow. If the reaction be positive, a deep blue color will appear either immediately or on allowing the tube to stand for a short time. After use wash the thimbles thoroughly in running water and then place them in boiling water for not over 15 seconds.

The reaction is carried out in the same way with the control tubes. Tube 2 should show a distinct positive reaction, while tube 3 should give a negative result. The tube with serum alone should show a negative reaction, but occasionally it is positive owing to the presence of a large amount of amino-acids in the serum as drawn. If the controls are all positive, the test is, of course, valueless, as some factor has been imperfectly controlled.

Bronfenbrenner's Modification.

Using the known property of antibodies of uniting with antigen at low temperatures, which prevent the activity of the complement and, also, exclude the activity of the proteolytic ferment, Bronfenbrenner adds to $\frac{1}{2}$ gram of placental tissue, contained in a glass tube, 1.5 c.c. of the suspected serum. Place this in the ice box over night. The next morning the serum is poured off, the tissue is placed in a centrifuge tube and washed, by centrifugation and decantation, until all traces of the serum are removed. The sensitized tissue is then placed in a dialyzing thimble and 1.5 c.c. of fresh male guinea-pig serum or normal human serum are added. Follow the usual technic of Abderhalden from this point. A positive ninhydrin reaction, under these conditions, is almost absolute evidence of pregnancy.

If the suspected serum, which has been in contact with the placental tissue in the ice box, be placed in a dialyzing thimble and kept at 37°C. for 18 hours, the dialysate will show a positive reaction due to the removal of the antitrypsin and the later self-digestion of the serum protein. The untreated serum, used as a control, should show no ninhydrin reaction.

¹ See Ruhemann, Jour. Chem. Soc., Trans., 1910, XCVII, 1438 and 2025; *Ibid.*, 1911, XCIX, 792; *Ibid.*, 1912, CI, 780; Abderhalden and Schmidt, Ztschr. f. physiol. Chem., 1911, LXII, 37; *Ibid.*, 1913, LXXXV, 143; Pearce, Jour. Am. Med. Assn., 1913, LXI, 1456; Warfield, *Ibid.*, 1914, LXII, 436; Halle, Löwenstein, and Pribram, Biochem. Ztschr., 1913, LV, 357; Neuberg, *Ibid.*, 1913, LVI, 500; Herzfeld, *Ibid.*, 1914, LIX, 249; Neuberg, *Ibid.*, 1914, LXVII, 56; Deetjen and Fränkel, Münch. med. Wchnschr., 1914, LXI, 466; Howe Biochem. Bull., 1914, III, 260; Emerson and Chambers, Jour. Lab. and Clin. Med., 1916, I, 752; Harding and Warneford, Jour. Biol. Chem., 1916, XXV, 319; Harding and MacLean, *Ibid.*, 337; Retinger, Jour. Am. Chem. Soc., 1917, XXXIX, 1059.

Value of the Test.

This test, applied by either one of the above methods, is extremely valuable. Abderhalden's results, which have been definitely confirmed (see bibliography at the end of this discussion), show that in the majority of cases in which the reaction was positive the patient has been proven to be pregnant; while negative reactions have been given only in non-pregnant cases. In other words, a negative result is definite, a point of distinction from a negative result with the complement-fixation test; while a positive result is evidence of the presence of specific elements within the blood (pregnancy), either at the time of the test or at least within a period which does not exceed two weeks (Schwarz). In the absence of a true pregnancy, hydatidiform mole and other pathologic conditions of the chorionic villi may be accountable for a positive reaction. A negative test should be considered as eliminating pregnancy.

As this reaction is evidence of pregnancy, in most cases giving positive results (an error of 10 per cent. being probable), it is especially interesting to find that it is positive from the middle of the second month of pregnancy on and disappears within 10 to 15 days after the termination of the pregnancy. It is, therefore, of great value in early diagnosis as well as in the diagnosis of a second pregnancy following so closely upon a previous one that other signs are not present. Differential diagnosis should be greatly improved by the use of this test and fewer laparotomies performed under the mistaken diagnosis of tumor. A further value of the test is found in the fact that medico-legal cases may be established on a firm basis of definite knowledge of the conditions present.¹

¹ For purposes of reference for those interested I give a fairly full bibliography of the subject. Abderhalden, Freund and Pincussohn, *Prak. Ergeb. d. Geburtsh. u. Gynäk.*, 1910, II, 367; Abderhalden, *Handb. d. biochem. Arbeitsmeth.*, 1911, V, 575; *Ibid.*, 1912, VI, 223; Abderhalden and Kiutsi, *Ztschr. f. physiol. Chem.*, 1912, LXXVII, 249; Abderhalden, *Ibid.*, 1912, LXXXI, 90; *Ibid.*, 1912, LXXXII, 109; *Münch. med. Wchnschr.*, 1912, LIX, 1190, 1305, 1939, and 2172; *Deutsch. med. Wchnschr.*, 1912, XXXVIII, 2160; Abderhalden and Weil, *Berl. Tierärztl. Wchnschr.*, 1912, XXVIII, 665; Frank and Heimann, *Berl. klin. Wchnschr.*, 1912, XLIX, 1706; Franz and Jarisch, *Wien. klin. Wchnschr.*, 1912, XXV, 1441; Veit, *Ztschr. f. Geburtsh. u. Gynäk.*, 1912, LXXII, 463; Petri, *Zentralbl. f. Gynäk.*, 1913, XXXVII, 235; Schwarz, *Interstate Med. Jour.*, 1913, XX, 195; Henkel, *Arch. f. Gynäk.*, 1913, XCIX, 56; Lindig, *Münch. med. Wchnschr.*, 1913, LX, 288; Abderhalden, *Ibid.*, 411 and 462; Jellinghaus and Losee, *Bull. Lying-in Hosp.*, New York, 1912, IX, 68; Gutman and Druskin, *Med. Record*, 1913, LXXXIV, 99; Engelhorn, *Münch. med. Wchnschr.*, 1913, LX, 587; Schlimpert and Hendry, *Ibid.*, 681; Freund and Brahm, *Ibid.*, 685; Freund, *Ibid.*, 700 and 763; Abderhalden, *Ibid.*, 701 and 763; Lindig, *Ibid.*, 702; Heimann, *Ibid.*, 915; Stange, *Ibid.*, 1084; Rübsamen, *Ibid.*, 1139; Schiff, *Ibid.*, 1197; King, *Ibid.*, 1198; Maccabruni, *Ibid.*, 1259; *Ann. di ostet.*, Milano, 1913, I, 486; Abderhalden, *Münch. med. Wchnschr.*, 1913, LX, 1386; Lampé and Papazolu, *Ibid.*, 1423 and 1533; Frank and Rosenthal, *Ibid.*, 1425; Lichtenstein, *Ibid.*, 1427; Heilner and Petri, *Ibid.*, 1530 and 1775; Steising, *Ibid.*, 1535; Frank, Rosenthal and Biberstein, *Ibid.*, 1594; Abderhalden and Weil, *Ibid.*, 1703; Schlimpert and Issel, *Ibid.*, 1758; Bruck, *Ibid.*, 1775; Goudsmit, *Ibid.*, 1775; Abderhalden and Schiff, *Ibid.*, 1923; Plotkin, *Ibid.*, 1942; Lampé and Fuchs, *Ibid.*, 2112; Tschudnowsky, *Ibid.*, 2282; Behne, *Zentralbl. f. Gynäk.*, 1913, XXXVII, 613; Parsamow, *Ibid.*, 934; Mayer, *Ibid.*, 1181; Porchownick, *Ibid.*, 1226; Schlimpert, *Berl. klin. Wchnschr.*, 1913, L, 1136; Rosenthal, *Ibid.*, 1149; Gottschalk, *Ibid.*, 1151; Veit, *Ibid.*, 1241; Aschner, *Ibid.*, 1243; Schäfer, *Ibid.*, 1605; Evler, *Ibid.*, 1606; Ebeler and Lohnberg, *Ibid.*, 1898; Williams and Pearce, *Gynec. and Obst.*, 1913, XVI, 411; McCord, *Ibid.*, 418; Judd, *Jour. Am. Med. Assn.*, 1913, LX, 1947; *Am. Jour. Med. Sc.*, 1913, CXLVI, 391; *Pari. Gazz. d. osp.*, 1913, XXXIV, 727; Ekler, *Wien. klin. Wchnschr.*, 1913, XXVI, 696; Jaworski and Szymanowski, *Ibid.*, 922; Daunay and Ecalle, *Compt. Rendu Soc. de biol.*, 1913, LXXIV, 1190; Sunde, *Norsk Mag. f. Laegevid.*, 1913, LXXIV, 1234; Schlimpert, *Brit. Med. Jour.*, 1913, II, 1003; *Deutsch. med. Wchnschr.*, 1913, XXXIX, 1225; Jonas, *Ibid.*, 1909; Naumann, *Ibid.*, 2086; Evler, *Med. Klin.*, 1913, IX, 1042 and 1086; Bauer, *Ibid.*, 1797; Abderhalden, *Monatschr., f. Geburtsh. u. Gynäk.*, 1913, XXXVIII, 24; Wolff,

2. Of Other Conditions.

The principles upon which Abderhalden's sero-diagnosis is supposedly based have been considerably altered by the newer researches. However, the method has found, and will continue to find, wide application in the diagnosis of varied conditions.¹ Although the basic idea of the presence of

Ibid., 394; Schwarz, Interstate Med. Jour., 1913, XX, 393; Jour. Am. Med. Assn., 1913, LXI, 484; Abderhalden, Gynäk. Rundschau, 1913, VII, 467; Decio, Ibid., 436; Ann. di ostet., 1913, I, 198; Abderhalden and Fodor, Ztschr. f. physiol. Chem., 1913, LXXXVII, 220; Abderhalden and Schiff, Ibid., 225 and 231; Gabastou and Widakowich, Semana Med., 1913, XX, 813; Scherer, Berl. klin. Wchnschr., 1913, L, 2183; Williamson, Jour. Obst. and Gynec. Brit. Emp., 1913, XXIV, 211; Werner and von Winiwarter, Wien. klin. Wchnschr., 1913, XXXI, 1841; Labusquière, Ann. de Gynec. et d'Obst., 1913, XL, 664; Fraenkel, Berl. klin. Wchnschr., 1913, L, 2287; Kabanoff, Med. Obozr., 1913, LXXX, 312; Sabin, Presse méd., 1913, XXI, 1016; Williams and Ingraham, Colorado Med., 1913, X, 364; Abderhalden, Münch. med. Wchnschr., 1913, LX, 2774; Lampé, Ibid., 2831; Meyer, Ibid., 2906; Wegener, Ibid., 1914, LXI, 15; Bronstein, Ibid., 74; Oeller, Stephan and Mayer, Ibid., 12 and 75; Behne, Zentralbl. f. Gynäk., 1914, XXXVIII, 74; Akimoto, Ibid., 81; Diner, New York Med. Jour., 1914, XCIX, 478; Holmes, Chicago Med. Recorder, 1914, XXXVI, 213, 475 and 593; Schwarz, Jour. Am. Med. Assn., 1914, LXIII, 371; Lindemann, Ztschr. f. d. ges. exper. Med., 1914, IV, 177; Guggenheimer, Ztschr. f. Chemotherapie., 1914, III (2Tl) 210; McLester, Am. Jour. Med. Sc., 1914, CXLVIII, 75; Pearce and Williams, Jour. Infect. Dis., 1914, XIV, 351; Pregl, Fermentforsch., 1914, I, 1; de Crinis, Ibid., 13; Abderhalden, Ibid., 20; Hirsch, Ibid., 33; Strauss, Ibid., 55; Paquin, Ibid., 58; Ross and Singer, Arch. Int. Med., 1914, XIV, 552; Ibid., 1915, XV, 724; Lange, Biochem. Ztschr., 1914, LXI, 193; Thar and Kotschnieff, Ibid., 1914, LXIII, 483; Salus, Ibid., 1914, LXV, 381; Parsamow, Ibid., 1914, LXVI, 269; Neuberg, Ibid., 1914, LXVII, 56; Hälsen, Ibid., 277; Fränkel, Ibid., 298; Thar and Kotschnieff, Ibid., 1915, LXIX, 389; Abderhalden, Deutsch. med. Wchnschr., 1914, XL, 268 and 401; Hirsch, Ibid., 270; Allmann, Ibid., 271; Pincussohn, Ibid., 425; Abderhalden, Ibid., 428; Lampé and Stroomann, Ibid., 635; Lampé, Ibid., 1213; Melikjanz, Ibid., 1369; Nieszytka, Ibid., 1519; Hirsch, Ibid., 1560; Otto and Blumenthal, Ibid., 1836; Eder, Ibid., 1838; Abderhalden, Münch. med. Wchnschr., 1914, LXI, 233 and 401; Voelkel, Ibid., 349; Singer, Ibid., 350; Lampé, Ibid., 463; Kämmerer, Clausz and Dieterich, Ibid., 469; Swart and Terwen, Ibid., 603; Abderhalden and Grigorescu, Ibid., 767; Abderhalden and Fodor, Ibid., 765; Schiff, Ibid., 768; Abderhalden, Holle and Strauss, Ibid., 804; Abderhalden and Paquin, Ibid., 806; Deetjen and Fränkel, Ibid., 826; Abderhalden and Wildermuth, Ibid., 862; Rosenthal and Biberstein, Ibid., 864; Abderhalden and Ewald, Ibid., 913; Lichtenstein and Hage, Ibid., 915; Freymuth, Ibid., 916; Griesbach, Ibid., 979; Abderhalden and Grigorescu, Ibid., 1209; Herzfeld, Ibid., 1503; Abderhalden, Ibid., 1879; Jaffé and Pribram, Ibid., 2125; Nieden, Ibid., 2200; Jaffé and Pribram, Ibid., 1915, LXII, 614; Puppel, Monatsschr. f. Geburtsh. u. Gynäk., 1914, XXXIX, 764; Fetzer, Ibid., 1914, XL, 598; Petri, Ibid., XLI, 309 and 388; Baumann, Ibid., 1915, XLII, 199; Wallis, Jour. Obs. and Gynec. of Brit. Emp., 1914, XXV, 53; Paine, Boston Med. and Surg. Jour., 1914, CLXX, 303; Ecalie, Arch. mens. d'Obs. et de Gynec., 1914, III, 257; Partos and d'Ernst., Ibid., 333; Jellinghaus and Losee, Am. Jour. Obs. and Dis. Women and Child., 1914, LXIX, 593; Kolmer and Williams, Ibid., 1915, LXXI, 899; Ibid., 1915, LXXII, 101; Grey, Bull. Johns Hopkins Hosp., 1914, XXV, 117; Echols, Wis. Med. Jour., 1914, XLIII, 1; Stoner and Skeel, Cleveland Med. Jour., 1914, XLIII, 392; Bullock, Lancet, II, 225; Gavronsky, Ibid., 1915, I, 119; Leitch, Brit. Med. Jour., 1914, II, 161 and 330; Hinselmann, Zentralbl. f. Gynäk., 1914, XXXVIII, 258; Kjaergaard, Ibid., 264; Schottlaender, Ibid., 425; Primars, Ibid., 438; Hussy, Ibid., 897; Gentili, Ibid., 1159; Saxl, Berl. klin. Wchnschr., 1914, LI, 824; Brieger and Schwalm, Ibid., 839; Ebeler and Löhnberg, Ibid., 1915, LII, 319; Abderhalden, Med. Klin., 1914, X, 665; Lampé and Peggger, Ibid., 725; Abderhalden and Grigorescu, Ibid., 728; Forster, Ibid., 772; von Graff and Saxl, Ibid., 1387; Lindstedt, Hygiea, 1915, LXXVII, 833; Goldstone, Med. Rec., 1914, LXXXVI, 67; Keitler and Lindner, Wien. klin. Wchnschr., 1915, XXVIII, 549; Goodman, Ann. Surg., 1915, LXI, 149; Kafka, Fermentforsch., 1915, I, 254; Ewald, Ibid., 315; Bunzel and Bloch, Münch. Med. Wchnschr., 1916, LXIII, 6; Rivas and Buckley, Jour. Med. Res., 1916, XXXIV, 297; Oppler, Biochem. Ztschr., 1916, LXXV, 211; Welker and Falls, Jour. Biol. Chem., 1917, XXXII, 509, 515, 519, and 521.

¹ See Wolff and Frank, Berl. klin. Wchnschr., 1914, LI, 875; Gumpertz, Beitr. z. Klin. der Tuberk., 1914, XXX, 201; Gwerder and Melikjanz, Münch. med. Wchnschr., 1914, LXI, 980; Ammenhäuser, Ibid., 2000; Schultz and Grote, Ibid., 2510; Baeslack, Jour. Am. Med. Assn., 1914, LXII, 1002; Ibid., 1914, LXIII, 559; Luxenburg, Med. Klin., 1914, X, 1104; Varney and Morse, Michigan State Med. Jour., 1914, XLII, 515; Hegner, Cor.-Bl. f. schweiz. Aerzte, 1914, XLIV, 1292; Vladesco and Popesco, Compt. rend. Soc. de biol., 1914, LXXVII, 586; Smith, Jour. Infect. Dis., 1915, XVI, 319; Falls, Ibid., 466; Voelkel,

specific ferments in the blood in pathologic conditions must, probably, be changed, yet the test appears to be somewhat specific in the sense that certain antibodies are probably present in the serum, which antibodies so sensitize the substrat that the antiproteolytic substance of the blood is withdrawn, thus leaving the protein of the serum free to undergo self-digestion by the normal proteolytic ferments of the blood. The possibility unquestionably exists that the use of a definite substrat (homologous in nature) may enable us to detect and differentiate pathologic processes much more sharply than has hitherto been the case. Although the specific nature of this test, as originally advocated by Abderhalden, has been attacked and shown to be dependent on other factors than those originally assumed, yet it is certain that there is much diagnostic value to the test, whatever may be its explanation. Considerable study must, however, be made before we are in a position properly to interpret our findings in all cases.

(a) Cancer.

In no other condition is a certain and constant diagnostic test so much to be desired as in cancer. Many methods have been advocated but Abderhalden's test is by far the most promising.

The technic of the test is as described above. As the protein material to be sensitized, cancerous tissue is employed, the material being cut into small bits and prepared exactly as is placental tissue. It would seem necessary to use homologous tissue in this work, that is carcinomatous or sarcomatous as the case may be. This point has not been definitely established but the results in any given case would, probably, be more specific. The dialyzation fluid is distilled water. Erpicum advises the use of a 2 per cent. solution of sodium fluorid as an antiseptic, but this is unnecessary if toluol is used freely as should be done. Sterility of all material and glass-ware is essential in this test.

The results reported have been more than encouraging in that benign growths seem to give, almost constantly, negative results while the malignant forms show an extremely large percentage of positive findings. The writer has used this test frequently and has corroborated his findings by pathological examination of the tissues removed at operation in many of the cases. In some of the cases the results have been far from satisfactory.¹ More detailed study is necessary before the value of this test in cancer is determined.

Münch. med. Wchnschr., 1914, LXI, 349; Wohl, Am. Jour. Med., Sc., 1915, CXLIX, 427; Oeri, Beitr. zur klin. der Tuberk., 1915, XXXIII, 211; Hippel, Fermentforsch., 1915, I, 233; Lampé and Cnopf, Ibid., 269; Abderhalden, Ibid., 351; Smith and Cook, Jour. Infect. Dis., 1916, XVIII, 14; Elsesser, Ibid., XIX, 655; Hüsey and Herzog, Arch. f. Gynäk., 1916, CV, 142; Datta, Rif. Med., 1916, XXXIII, 204; Izabolinsky, Russk. Vrach, 1916, XV, 808.

¹ See Abderhalden, Münch. med. Wchnschr., 1913, LX, 2385; Labbé, Gaz. méd. de Nantes, 1913, 2 me. S., XXXI, 461; Frank and Heimann, Berl. klin. Wchnschr., 1913, L, 631; Markus, Ibid., 776; Münzer, Ibid., 777; Epstein, Wien. klin. Wchnschr., 1913, XXVI, 649; Deutsch and Köhler, Ibid., 1361; Erpicum, Bull. de l'Acad. roy. de méd. de Belg., 1913, XXVII, 624; Ludke, Gaz. d. hôp., Paris, 1913, LXXXVI, 1064; von Gambaroff, Münch. med. Wchnschr., 1913, LX, 1644; Brockman, Lancet, 1913, II, 1385; Abderhalden, Deutsch. med. Wchnschr., 1913, XXXIX, 2391; Hara, Ibid., 2559; Fried, Münch. med. Wchnschr., 1913, LX, 2782; Schwarz, Am. Jour. Obst., 1914, LXIX, 54; Ball, New York Med. Jour., 1913, XCVIII, 1249; Jour. Am. Med. Assn., 1914, LXII, 599; Halpern.

(b) Dementia Præcox.

In the realm of nervous disorders differential diagnosis is not always as exact as could be desired. Abderhalden's test has been applied by many in such cases with excellent results. In this work the selection of the proper material for the substrat seems to be the matter of greatest importance. It is probable that testicular tissue should be used for male patients and ovarian tissue for female sufferers, although cortical tissue and thyroid have been used by some but positive results with the latter are few. The adoption of cortical tissue is highly improbable as differential diagnosis is not subserved by its use. General paralysis, epilepsy with dementia, and manic-depressive insanity all respond promptly to brain tissue but very rarely to testicular or ovarian material. A further point is that all syphilitic and parasyphilitic disorders seem to cause hydrolysis of cortical brain tissue but not of other organs. The antibodies found in the serum of these patients are, therefore, more or less specific, as a reaction with testicular or ovarian tissue is almost positive evidence of dementia præcox.¹

E. Herman-Perutz Reaction.

As the Wassermann test for syphilis is so complex and requires so much experience for its proper performance and interpretation, attempts have been made to find a serum reaction for syphilis which could be used by the general worker and which, at the same time, would give results comparable to those of the Wassermann test. Such a test is that of Herman and Perutz,² which is a

Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1914, XXVII, 340; Piorkowski, Berl. klin. Wehnschr., 1914, LI, 254; Fränkel, Ibid., 356; Fasiani, Wien. klin. Wehnschr., 1914, XXVII, 267; Manoiloff, Ibid., 269; Oeller and Stephan, Münch. med. Wehnschr., 1914, LXI, 579 and 583; Schawlow, Ibid., 1386; Weinberg, Ibid., 1617, 1685 and 1732; Fränkel, Deutsch. med. Wehnschr., 1914, XL, 589; Hara, Ibid., 1258; Cytronberg, Mitt. a. d. Grenzgeb. der Med. u. Chir., 1914, XXVIII, 243; Heimann and Fritsch, Arch. f. Chir., 1914, CIII, 659; Lanzarini, Gazz. d. osp., 1914, XXXV, 1057; Trubina, Deutsch. Zeitsch. f. Chir., 1914, CXXXI, 520; Fulchiero, Rif. med., 1914, XXX, 1261; Van Slyke and Vinograd, Proc. Soc. Exper. Biol. and Med., 1914, XI, 154; Dick, Jour. Infect. Dis., 1914, XIV, 242; Kahn, Arch. Diag., 1914, VII, 356; Biehn, Ill. Med. Jour., 1915, XXVII, 206; Goodman, Surg., Gyn. and Obs., 1914, XIX, 797; Levin, New York Med. Jour., 1914, C, 621; Bullock, Lancet, 1915, I, 223; Goodman and Berkowitz, Surg., Gyn. and Obs., 1915, XXI, 463; Ball, Jour. Am. Med. Assn., 1914, LXII, 590; Ibid., 1914, LXIII, 1169; Lowy, Ibid., 1915, LXIV, 1559; Levin and Van Slyke, Ibid., 1915, LXV, 945; Berghausen, Interstate Med. Jour., 1915, XXII, 228; Drummond, Biochem. Jour., 1916, X, 473; de Crinis and Mahnert, Fermentf., 1918, II, 103.

¹ See Fauser, Deutsch. med. Wehnschr., 1913, XXXIX, 304; Wegener, Münch. med. Wehnschr., 1913, LX, 1197; Schulz, Deutsch. med. Wehnschr., 1913, 39, 1399; Lampé, Ibid., 1774; Bundschuh and Roemer, Ibid., 2029; Fischer, Ibid., 2138; Fuchs, Münch. med. Wehnschr., 1913, LX, 2230; Binswanger, Ibid., 2321; Theobald, Med. Klin., 1913, IX, 1850; Berl. klin. Wehnschr., 1913, L, 2180; Beyer, Münch. med. Wehnschr., 1913, LX, 2450; Kafka, Med. Klin., 1914, X, 153; Fauser, Münch. med. Wehnschr., 1914, LXI, 126; Kastan, Deutsch. med. Wehnschr., 1914, XL, 319; Arch. f. Psychiat., 1914, LIV, 928; Grigorescu, Med. Klin., 1914, X, 418; Neue, Ibid., 1217 and 1259; Simon, Jour. Am. Med. Assn., 1914, LXII, 1701; Sterne, Ill. Med. Jour., 1914, XXVI, 327; Gehrman, Ibid., 335; Holmes, Ibid., 332; New York Med. Jour., 1914, XCIX, 567; Chicago Med. Recorder, 1914, XXXVI, 644; Ibid., 1915, XXXVII, 88; Bouman and Hasselt, Nederl. Tijdschr. v. Geneesk., 1915, I, 423; Mayer, Münch. med. Wehnschr., 1915, LXII, 580; Sioli, Arch. f. Psychiat. u. Nervenkr., 1914, LV, 241; Obregia and Pitulesco, C. R. soc. biol., Paris., 1914, LXXIV, 47; Kafka, Münch. Med. Wehnschr., 1915, LXII, 1316 and 1355; Wegener, Fermentforsch., 1915, I, 210; Parhon and Parhon, Ibid., 311; Colton, White and Stevenson, Jour. Nerv. and Ment. Dis., 1915, XIII, 259; Jour. Nerv. and Ment. Dis., 1916, XLV, 144; Singer and Quantz, Arch. Int. Med., 1916, XVIII, 520; Falls, Jour. A. M. A., 1916, LXVI, 22; Retinger, Arch. Int. Med., 1918, XXII, 234.

² Med. Klin., 1911, VII, 60. Landau (Wien klin. Wehnschr., 1913, XXVI, 1702; see, also, Presse méd., 1914, XXII, 335, and Riv. osped., 1914, IV, 641) has introduced a test for

modification of an older test of Porges. Its simplicity should recommend it, especially as its results are usually definite and easily interpreted.

syphilis, using as his reagent a 1 per cent. solution of iodine in carbon tetrachloride. To 0.2 c.c. of fresh serum he adds 0.1 c.c. of the above reagent, shakes the tube and allows the mixture to stand for four hours. Positive results are shown by a clear yellow serum above the decolorized reagent, indicating the binding of the iodine probably by the lipoids. If the serum is an opaque grayish white, the reaction is negative. While this test gives results in many cases which parallel those of the Wassermann test, yet it shows a large percentage of positive results in non-syphilitic cases. Further, a negative Landau test is of little value as many known syphilitics give negative tests. This test has very little diagnostic value. See Golay, *Rev. méd. de la Suisse romane*, 1914, XXXIV, 571; Villaret and Pierret, *Presse méd.*, 1914, XXII, 582; Correa, *Brazil-Medico*, 1914, XXVIII, 395; Kolmer, *Jour. Am. Med. Assn.*, 1915, LXIV, 1461 and 1966; Stillians, *Ibid.*, 1964; Capello, *Gazz. d. osp.*, 1915, XXXVI, 423; Recupero, *Ibid.*, 854; Chiaravallotti, *Pediatrics*, 1915, XXIV, 120. Bruck (Münch. med. Wchnschr., 1917, LXIV, 25) has introduced a test, which depends upon the difference in solubility in distilled water of a precipitate formed by dilute nitric acid in the case of syphilitic sera, as compared with normal sera. In this test 0.5 c.c. of clear corpuscle-free serum, which has not been heated, is placed in a large test-tube and 2 c.c. of distilled water added and the mixture gently mixed by shaking. The exact time being noted with a watch, 0.3 c.c. of 25 per cent. nitric acid (sq. g. 1149, made by diluting 100 c.c. of C. P. Nitric acid with 225 c.c. of distilled water) is added and the mixture gently shaken. This is allowed to stand at room temperature for exactly 10 minutes. To the material in the tube, which should now contain a white precipitate, is added exactly 16 c.c. of distilled water. The tube is closed with the finger and the contents mixed by inverting the tube three times, carefully so as not to cause foam. Allow this to stand 10 minutes and mix as before, after which the tube is set aside for $\frac{1}{2}$ hour, when the result of the test is read. With normal serum, the precipitate is supposed to dissolve completely, the water being clear or slightly opalescent; with syphilitic sera, small flakes are formed, which show little tendency to dissolve and which settle out on allowing to stand. A well defined sediment is marked +; a slight one, \pm ; none, —. While this reaction parallels the Wassermann reaction in about 75 per cent. of cases of known syphilis, yet the results are not sufficiently specific to warrant more than a confirmatory evidence. See Smith and Solomon, *Boston Med. and Surg. Jour.*, 1917, CLXXVII, 321; Stillians, *Jour. A. M. A.*, 1917, LXIX, 2014; Toyama and Kolmer, *Jour. Cutan. Dis.*, 1918, XXXVI, 429; Boruttau, *Ztschr. f. angew. Chem.*, 1918, XXXI, 65; Terada, *Kitasato Arch. f. Exper. Med.*, Tokyo, 1919, III, 123. Sachs and Georgi (Sachs, *Ztschr. f. Immunität*, 1917, XXVI, 451; Georgi, *Med. Klinik*, 1918, No. 33); *Biochem. Ztschr.*, 1919, XCIII, 16.

Sachs and Georgi, *Münch. med. Wchnschr.* 1920, LXVII, 66) have originated a flocculation test for syphilis based on the action of a cholesterinized antigen upon syphilitic serum. The antigen consists of an alcoholic extract of normal beef heart to each 100 c.c. of which 200 c.c. of alcohol and 13.5 c.c. of a 1 per cent. solution of cholesterin are added. For use this antigen is diluted 1:5 with normal saline. Dilute 1 c.c. of the clear serum to 10 c.c. with physiologic salt solution and add 0.5 c.c. of the diluted antigen and let the tubes stand at room temperature or in the incubator for 24 to 48 hours. Normal serum shows no precipitate, while syphilitic serum shows more or less flocculation in a large percentage of cases. Although this test parallels the Wassermann test in a large percentage of known cases of syphilis and shows about the same variance in negative cases, yet it will hardly be relied upon to the exclusion of the more exact complement fixation method. See Reich, *Deutsch. med. Wchnschr.*, 1919, XLV, 181; Merzweiler, *Ibid.*, 1273; Felke and Wetzell, *Münch. med. Wchnschr.*, 1919, LXVI, 1347; Raabe, *Berl. klin. Wchnschr.*, 1919, LVI, 1012; Wolfenstein, *Ibid.*, 1110; Galli-Valerio, *Correspond.-Bl. f. Schweiz. Aetzr.*, 1919, XLIX, 1977; Messerschmidt, *Deutsch. med. Wchnschr.*, 1920, XLVI, 150; Schönfeld, *Münch. med. Wchnschr.*, 1920, LXVII, 399; Hinzelmann, *Ibid.*, 402; Pesch, *Ibid.*, 1232; Somogyi, *Ibid.*, 1233; Bok, *Nederl. Tijdschr. v. Geneesk.*, 1921, I, 1328; Dekenga and Plantenga, *Ibid.*, 1631; Fleisch, *Schweiz. med. Wchnschr.*, 1920, L, 466; Neukirch, *Ztschr. f. Immunität*, 1920, XXIX, 498; Meyeringh, *Ibid.*, XXX, 51; Scheer, *Ibid.*, 178; Wendtlandt, *Ibid.*, 202; Armmenhausser, *Centraltbl. f. Bakt. u. Parasitenkde.*, 1920, LXXXIV, 521; Hull and Faught, *Jour. Immunol.*, 1920, V, 521; Pincherle, *Policlinico*, 1920, XXVII, 979; Marcora, *Ibid.*, 1107; Logan, *Lancet*, 1921, I, 14; Levinson and Peterson, *Arch. Dermatol. & Syph.*, 1921, III, 286; Parker and Haigh, *Ibid.*, IV, 66; D'Aunoy, *Jour. Med. Res.*, 1921, XLII, 339; Levinson, *Am. Jour. Syph.*, 1921, P, 414; Stühmer and Merzweiler, *Deutsch. med. Wchnschr.*, 1921, XLVII, 559; Sachs and Georgi, *Med. Klinik*, 1921, XVII, 987; Tamiguchi and Yoshinare, *Brit. Med. Jour.*, 1921, II, 239; Niederhoff, *Münch. med. Wchnschr.*, 1921, LXVIII, 330; Epstein and Paul, *Arch. f. Hyg.*, 1921, XC, 98; Yoshinare, *Jour. Path. & Bact.*, 1921, XXIV, 358; Kingsbury, *Lancet*, 1921, II, 799; Scalas, *Rif. Med.*, 1921, XXXVII, 1166; Wolf, *Schweiz. med. Wchn.*, 1922, LII, 118; Gaetgens and Salvioli, *Med. Klinik*, 1922, XVIII, 179.

For this test two solutions are used.

Solution A.

Sodium glycocholate,	2.00 grams
Cholesterin,	0.40 gram
95 per cent. alcohol,	100.00 c.c.

At the time of making the test, this solution is diluted with distilled water in proportion of 1 to 20.

Solution B.

A 2 per cent. aqueous solution of sodium glycocholate.

This solution must be made up fresh at the time of making the test, as it is not permanent. Further, the solution is not a perfect one so that the bottle should be shaken before using.

Technic.

Withdraw the blood as for the Wassermann test. As only a small amount is needed, puncture of the ear or finger is usually sufficient. Collect the blood in a sterile tube, allow it to clot and centrifuge. The serum must show no hemolysis. Pipet off the clear serum. Although Herman and Perutz believed the test was better if the serum was inactivated by heating to 56°C. for one-half hour, later work has shown that this is not essential.

To 0.4 c.c. of the clear serum add 0.2 c.c. of the 1 to 20 dilution of solution A and 0.2 c.c. of solution B. Shake thoroughly, plug the tubes with cotton and allow them to stand not longer than 18 to 24 hours at room temperature. On examining the tubes, a positive result is indicated by a flocculent precipitate, while a negative result is shown by no precipitation. The results may be expressed as +, ++, +++ depending on the degree of precipitation. This gives the results so that comparison with the Wassermann may be made easily.

This test practically parallels the Wassermann test, except that in secondary syphilis the results are not quite so uniform with the former. The percentage of variation is greater than with the Wassermann test, so that our interpretation of the result would be guarded. In other words, a positive result indicates syphilis, while a negative result does not exclude syphilis. This reaction should find a place in the laboratory of every general worker who does not have facilities for performing the Wassermann test, but should not be relied upon to the exclusion of the Wassermann test.¹

F. Coagulo Reaction.

This reaction, originated by Hirschfeld and Klinger,² is based on the

¹ See Gammeltoft, *Hospitaltid.*, 1912, V, 471; *Deutsch. med. Wehnschr.*, 1912, XXX, VIII, 1934; Jensen and Feilberg, *Hospitaltid.*, 1912, V, 493; Feilberg, *Ibid.*, 973; Ipsen and Helweg, *Ibid.*, 1341; Thomsen and Boas, *Ibid.*, 1152; Jensen, *Ibid.*, 1516; Pontoppidan, *Ugesk. f. Laeger*, 1912, LXXIV, 1377; Jensen and Feilberg, *Berl. klin. Wehnschr.*, 1912, XLIX, 1086; Lade, *Deutsch. med. Wehnschr.*, 1913, XXXIX, 693; Kallos, *Ibid.*, 1885; Leschly and Boas, *Hospitaltid*, 1913, VI, 640; Thomsen and Boas, *Ztschr. f. Immunitätsforsch.*, 1913, XVI, 430; Olitsky and Olmstead, *Jour. Am. Med. Assn.*, 1914, LXII, 293; Bing and Schmitz, *Ugesk. f. Laeger*, 1916, LXXVIII, 1110; Perutz, *Wien. klin. Wehnschr.*, 1916, XXIX, 1619; MacCann, *Jour. Lab. and Clin. Med.*, 1919, IV, 742.

² *Deutsche med. Wehnschr.*, 1914, XL, 1607; *Sem. Med.*, 1914, XXXIV, 361; *Münch. med. Wehnschr.*, 1914, LXI, 2093; see, also, Fränkel and Thiele, *Ibid.*, 2095; Cole and Chin, *Arch. Int. Med.*, 1915, XVI, 880; Brandt, *Deutsche med. Wehnschr.*, 1915, XLI, 915.

observation that syphilitic serum delays or even prevents the coagulation of recalcified oxalate plasma by inhibiting the production of thrombin through interference with the activity of thrombokinase. It is to be recalled that coagulation of the blood is due to the formation of fibrin from fibrinogen through the influence of a ferment, thrombase (thrombin), which is present in the leucocytes and blood plates (and may be obtained *in vitro* by extraction of almost all tissues with alcohol) in the form of prothrombase. This latter zymogen is changed, through the influence of ionized calcium compounds, into thrombin. It has, further, been shown that thrombin consists of two substances: namely, (1) the serozyme or thrombogen, a protein constituent of the plasma and (2) the cytozyme or thrombokinase, which belongs to the group of lipoids (largely lecithins). This cytozyme, therefore, it will be seen, is essential to the formation of a coagulum. A simple procedure would, of course, be to test a fluid by the addition of fibrinogen solutions. However, such solutions are unstable, so that, as Bordet and Delange¹ have shown, we may employ oxalate plasma instead. This latter has the advantage that it will keep for some time and that its addition prevents any further formation of thrombin, as the calcium of the serum is precipitated by the oxalic acid radical in the form of calcium oxalate.

In the performance of this test there are, so to speak, three phases, which may be outlined as follows: (1) Mix the heated serum with varying dilutions of the cytozyme (alcoholic extract of tissue, preferably human heart) and allow the mixtures to stand for $\frac{1}{2}$ to 1 hour to permit the inactivation of the cytozyme by the serum; (2) add solution of calcium chlorid and the serozyme (fresh plasma or recalcified oxalate plasma) and allow to stand for fifteen minutes to permit the production of thrombin, providing, of course, that cytozyme is available, the amount of thrombin formed being in direct ratio to the amount of cytozyme present; (3) add oxalate plasma to test for the presence and amount of thrombin and time the reaction of coagulation.

Reagents Necessary.

1. Oxalate Plasma.—Into a 100 c.c. volumetric flask place 10 c.c. of a 1 per cent. solution of sodium oxalate to which has been added $\frac{1}{20}$ volume of 10 per cent. solution of sodium chlorid (this latter is added to render the solution isotonic for the blood, according to the suggestion of Uemura²). With a short, stout, sterilized needle obtain the blood from the external jugular vein of a sheep or goat, collecting slightly more than 100 c.c. Allow a few c.c. of this blood to flow out of the syringe and add the remaining blood to the fluid in the flask, up to the 100 c.c. mark. Before drawing the blood it is wise to heat the solution in the flask to 40°C. and moisten the interior of the flask with this warm oxalate solution to prevent the condensation of steam from the warm blood on the wall of the flask, as this may produce some hemolysis. During the addition of the blood to the solution in the flask, shake gently in order to mix thoroughly and prevent coagulation, as every-

¹ Ann. de l'Inst. Pasteur, 1912, XXVI, 657 and 737; Ibid., 1913, XXVI, 341.

² Am. Jour. Med. Sc., 1917, CLIV, 533.

thing possible must be done to prevent the formation of thrombin at this stage. The oxalated blood is now poured into centrifuge tubes, which have been previously rinsed with warm physiologic salt solution, and centrifuged for 15 to 20 minutes; the supernatant fluid is then transferred to fresh centrifuge tubes and again centrifuged for 30 minutes at high speed to remove the platelets. The resulting plasma should be absolutely clear and free from hemoglobin; if colored at all, it should be nothing more than a yellow. This will keep in the ice-box for one to two weeks. When used in the test, this oxalate plasma is diluted as follows: 1 part oxalate plasma; 5 parts physiologic salt solution; and $\frac{1}{2}$ part of 1 per cent. sodium oxalate solution. Should coagula appear in this plasma after standing for a few days, these may be filtered off through sterile cotton wool.

2. *The Serozyme*.—This is prepared from the above oxalate plasma as follows: To 50 c.c. of this oxalate plasma add 5 c.c. (one-tenth volume) of 1 per cent. calcium chlorid solution; mix well and place in the incubator for at least 15 minutes. The plasma should have coagulated by this time, but, if it has not done so or has only partially completed the process, add a few more drops of the calcium chlorid solution. Plasmas, which have been kept for some time, often will not coagulate at all, or, at least, very slowly; such can not be used for the preparation of the serozyme but may be satisfactory where the oxalate-plasma, itself, is directed. When the coagulum has formed, grasp it with a long forceps and express the serozyme by pressing with a wringing movement. Should a second coagulum appear, this should be removed in the same manner. Freshly prepared serozyme may contain traces of thrombin, so should not be used for testing purposes until at least one or two hours have elapsed, in order to be certain that all tendency to coagulate is passed. When used in the test, this serozyme is diluted with five volumes of physiologic salt solution and employed in the proportion of 0.5 c.c. per tube. Before use a preliminary test should show that it is satisfactory, that is that it contains neither cytozyme nor thrombin. For every test, it should be controlled in this direction, by placing 0.5 c.c. of the diluted serozyme in a tube with 1 c.c. of the calcium chlorid solution, without any cytozyme, and adding 1 c.c. of oxalate plasma. This mixture should not coagulate, at least not until the following day. According to Uemura, weak serozyme may be considerably improved by diluting it with 10 volumes of distilled water and allowing it to stand for several hours; to the cloudy mixture is added sufficient 10 per cent. sodium chlorid to make it contain 0.8 per cent. NaCl, after which it may be employed in doses of 1 c.c.

3. *Calcium Chlorid Solution*.—This is prepared by mixing 5 c.c. of a 1 per cent. solution of calcium chlorid with 95 c.c. of physiological salt solution.

4. *The Cytozyme*.—This is an alcoholic extract of tissues which contains, especially, the lecithin group of lipoids. These may be the same alcoholic extracts that are used as antigens in the Wassermann test, the extract of human, beef, or guinea-pig heart being apparently preferable. The concentration and effectiveness of this extract should be such that, diluted 20 to 40 times and tested as given below, 0.1 c.c. causes the oxalate plasma to

coagulate in one to three minutes. To test its strength proceed as follows: In two test-tubes place 0.1 c.c. of 1:20 and 1:40 dilutions of this extract in physiologic salt solution respectively, add to each tube 1 c.c. of the calcium chlorid solution, 0.5 c.c. of the serozyme solution and 1 c.c. of the oxalate plasma dilution. Coagulation should result in 1 to 3 minutes. In the actual performance of the test, four dilutions are employed in constant dose of 0.1 c.c.: namely, 1:40; 1:80; 1:160; 1:320. The coagulation time will, of course, differ with each dilution.

5. *The Serum*.—This is collected as for the Wassermann test. It must be clear and absolutely free of corpuscles and dissolved hemoglobin. Uemura has shown that admixture of small quantities of hemoglobin would affect the sera in such a way as to diminish their inhibition of coagulation. The serum resulting from spontaneous coagulation is very likely to contain cytozyme. For this reason, before use in the test, the sera should be heated for 1 hour, the optimum temperature being 56 to 58°C., as Toyama¹ has shown. Specimens received through the mails may contain a relatively large amount of this cytozyme and may require heating to 60 for 1 hour, but the results obtained with such heated sera may show false positive reactions

Technic.

Before beginning the actual test, it is assumed that the reagents have all been tested as to their activity. The test outlined above under Cytozyme, will show, of course, that these are active as far as the actual coagulation is concerned. If the coagulation does not result within three minutes, the trouble is usually due to defective serozyme or cytozyme. Further, the reagents should be tested against known positive and negative syphilitic sera, to show that the reagents, as prepared, do produce a delay in coagulation of the syphilitic as compared with the normal serum.

The test is conducted as follows:

1. For each serum to be tested arrange 5 small, clean, sterile test-tubes in a row; into each of the first four place 0.1 c.c. of 1:40, 1:80, 1:160, and 1:320 salt solution emulsions of the alcoholic extract of human heart (cytozyme), respectively. The fifth tube is the serum control and receives 0.1 c.c. of salt solution.

2. To each of the 5 tubes add 0.1 c.c. of heated serum; mix well and stand aside at room temperature for an hour.

3. To each tube add 1 c.c. of the calcium chlorid solution and 0.5 c.c. of the diluted serozyme solution; mix well and stand aside at room temperature for 15 minutes.

4. To each tube add 1 c.c. of the diluted oxalate plasma, recording accurately with a watch the time when oxalate plasma was added; mix well and examine each tube every minute for coagulation.

5. *Controls*.—The fifth tube of each series is the serum control and should remain fluid for several hours at least and, usually, until next day or even indefinitely. Should coagulation occur in this tube, a fresh specimen of the serum should be heated to 60 for an hour and the test repeated. Each dilu-

¹ Am. Jour. Syph., 1919, III, 6.

tion of cytozyme in dose of 0.1 c.c. should be set up without serum (substituting 0.1 c.c. of normal salt solution) and should show coagulation in from 1 to 6 minutes, the higher dilutions showing, of course, the longer period. Further, positive and negative syphilitic sera should be run through at the same time.

An accurate record of time in minutes must be kept of each tube of the series and the serum controls, until the tubes of normal serum containing cytozyme and the tubes containing all reagents except serum show coagulation; after this each tube should be examined at close intervals. The serum controls should show no coagulation. Normal or nonsyphilitic sera usually coagulate within 10 minutes, but this interval may be shorter or longer (an hour or more) depending on the quality of the cytozyme, serozyme, and oxalate plasma. Strongly syphilitic sera delay coagulation for considerably longer periods; weakly syphilitic sera delay coagulation only slightly beyond the time required for normal serum. *No standard time can be laid down for the coagulation of normal or syphilitic serum, as this varies with each new lot of reagents.* Coagulation is indicated by the development of a firm jelly-like coagulum in the tubes. The results are expressed, as given by Kolmer and Toyama,¹ as \pm , +, and ++. The first indicates slight delay in coagulation; the second a delay of 5 to 10 minutes; and the third a longer delay. For no coagulation the fluid state may be indicated by fl.

G. Tests before Transfusion.

As transfusion of blood is becoming a matter of more or less frequent occurrence, it is advisable to test the blood for *isoagglutinins* and *isohemolysins* in every possible case.² "That donor should be chosen whose blood shows no inter-agglutination or hemolysis with the patient's serum and corpuscles. If such a donor cannot be obtained, it is safer to use a person whose serum is agglutinative toward the patient's cells than one whose cells are agglutinated by the patient's serum" (Kolmer).

Technic.

Two or three c.c. of blood are obtained from each donor by the usual serological method. Place 0.5 c.c. at once in a centrifuge tube containing 5 c.c. of a 1 per cent. solution of sodium citrate in normal salt. Place the remainder of the blood in a small dry test-tube and allow the serum to separate. Obtain 3 or 4 c.c. of blood from the recipient in the same manner and follow the same procedure with this blood as with that of the donors.

Centrifuge the sodium citrate tubes, pipet off the supernatant fluid and wash the cells again with normal salt solution. Repeat if necessary in order to free the cells from every trace of serum. After the final washing, add enough normal salt solution to the cells to make a total volume of 5 c.c.

Centrifuge the serum tubes and separate the clear serum. This serum

¹ Am. Jour. Syph., 1918, II, 505.

² Hepp (Jour. Exp. Med., 1920, XXXI, 313) has shown that during the 1st month of life isoagglutination is rarely present but that after 1 year the group is usually established, and after 2 years is always present. See, also Jones, Am. Jour. Dis. Child., 1921, XXII, 586.

should show no trace of hemolysis. The following series of tests should then be set up. In this work the blood should not be allowed to stand for a period of time before the elements are separated, and the actual tests should be begun within 24 hours of the withdrawal of the blood. Use small sterile test-tubes about 8 by 1 cm.

1. Four drops of donor's serum + 1 drop of recipient's red-cell emulsion.
2. Four drops of recipient's serum + 1 drop of donor's red-cell emulsion.
3. Control: Four drops of donor's serum + 1 drop of donor's red-cell emulsion.
4. Control: Four drops of recipient's serum + 1 drop of recipient's red-cell emulsion.
5. Control: One drop of donor's red-cell emulsion + 4 drops of normal salt solution.
6. Control: One drop of recipient's red-cell emulsion + 4 drops of normal salt solution.

One c.c of normal salt solution is added to each tube and the tubes are shaken and placed in the incubator for two hours, being inspected about every one-half hour. Agglutination in tubes 1 and 2 may be recognized macroscopically by the clumping of the red cells. Hemolysis is noted as in previous tests. If there is any question about hemolysis, let the tubes stand in the ice box over night and read them as in the complement-fixation tests. Tubes 3, 4, 5, and 6 should show no hemolysis or agglutination.

Besides the above tests, it is advisable to test the donors with the Wassermann test. Further, immediate members of the patient's family offer the least risk in transfusion and should be obtained whenever possible.

The above macroscopic method, while reliable, is, nevertheless, rather cumbersome and time consuming. For these reasons, Rous and Turner¹ introduced a rapid and simple method, which involved the collection of the blood in a pipet holding 0.25 c.c. of fluid, the mixing of the recipient's blood and that of the donor in Wright's pipets, and the microscopic examination of the mixtures for agglutination, in a few minutes if there is an emergency, or at the end of fifteen minutes, if time is not a factor. With this technic, the difficulty not infrequently arises that clots may form and interfere with the test. Minot² has brought out a modification of this test, which is more simple and does away with the collection in the pipet, thus obviating the difficulty with the original technic. This latter worker collects the specimens as follows: Place 3 or 4 drops of a 1.5 per cent. sodium citrate solution in 0.9 per cent. physiological salt in a small test-tube. Collect, from a prick in the finger or ear, in this tube 9 drops of blood from the recipient and one from the donor and in another tube collect 9 drops from the donor and one from the recipient. Mix by shaking, allow to stand for 15 minutes, and examine microscopically for agglutination. This method yields as accurate results as those mentioned above and is much more easily carried out, where the reciprocal study of recipient's and donor's blood is undertaken.

¹ Jour. A. M. A., 1915, LXIV, 1980.

² Boston Med. and Surg. Jour., 1916, CLXXIV, 667.

Grouping of Bloods.

Through the work of Landsteiner and others it has been established that human blood has two different isoagglutinins. These have been designated with the capital letters A and B. The elements, which are agglutinable are resident in the corpuscles and have been identified by the letters a and b. It is self evident that a blood can not contain either agglutinin A or B and its corresponding agglutinable substance a or b, as this would lead to agglutination of the blood corpuscles by its own plasma.¹ However, there are four different combinations of these elements, which are possible and which are shown to exist in the blood. This has lead to the study of the grouping of the bloods of individuals, based upon their agglutinin content as well as upon the power of their corpuscles to be agglutinated by other sera. While the early workers, as Landsteiner, Descatello and Sturli, and Hektoen, classified the blood in three groups, today we recognize 4 such groups and follow the classification of Jansky or of Moss. These two latter systems of grouping the bloods differ merely in the placing of the most common group. Jansky styles this group I, while Moss classifies it as group IV. In other words, groups I and IV of Jansky's classification become Groups IV and I of the Moss grouping. Groups II and III of each classification remain the same. While the Jansky grouping has priority, yet it has not been so widely adopted as the Moss system. In this discussion we will follow the Jansky grouping,² inviting attention to the points mentioned above for those who prefer the Moss method. The value of this study of grouping of blood is shown when blood is to be selected for transfusion. If the group to which a recipient belongs is known or determined, as outlined below, a proper blood may be selected from a list of donors, which may be available and which belong to the same group as the recipient.

The four possibilities of combination of the agglutinating and agglutinable elements mentioned above are in the Jansky classification as follows:

Group I.	{ Plasma contains agglutinins A and B. Corpuscles contain no agglutinable elements.
Group II.	{ Plasma contains agglutinin A. Corpuscles contain b.
Group III.	{ Plasma contains agglutinin B. Corpuscles contain a.
Group IV.	{ Plasma contains no agglutinins. Corpuscles contain a and b.

From a study of the above groupings, it naturally follows that:

(a) the plasma or serum of Group I will agglutinate the corpuscles of Groups II, and III and IV while the corpuscles of this group are not agglutinable by the sera of any of the other groups.

(b) the plasma or serum of Group II will agglutinate the corpuscles of

¹ However cases of autohemo-agglutination do occur as reported by Clough and Richter (Bull. John. Hopks Hosp., 1918, XXIX, 326) and by Kligler (Jour. Am. Med. Assoc., 1922, LXXVIII, 1105).

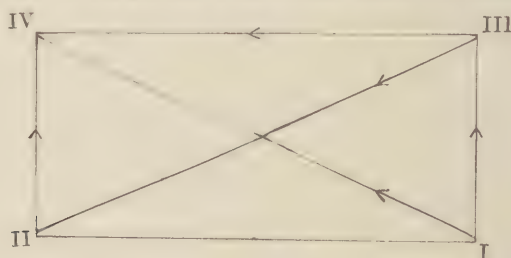
² See report of special committee, Jour. Am. Med Assoc., 1921, LXXVI, 130.

Groups III, and IV, while the corpuscles of this group are agglutinable by sera of Groups I and III.

(c) the plasma or serum of Group III will agglutinate the corpuscles of Groups II and IV, while the corpuscles of this group are agglutinable by sera of I and II.

(d) the plasma or serum of an individual of Group IV will not agglutinate the corpuscles of any other group and that the corpuscles of this group are agglutinable by the sera of all other groups;

The above points, which characterize the serum and corpuscles of each of the 4 blood groups, as far as their agglutinating actions is concerned, may be noted from the following graphic representation, which is taken from Sanford.¹ In this figure, the corpuscles of the various groups are agglutinated by the sera of the groups from which the arrow's head.



The proportional representation of these four groups among human individuals, as far as investigated cases indicate, is, according to Moss, as follows: Group I, 10 per cent.; Group II, 40 per cent.; Group III, 7 per cent.; Group IV, 43 per cent. In the Jansky classification, Group I represents the largest group.²

Methods of Determining Groups.

In any method for such study one should have on hand either sera or corpuscles of Groups II and III, at least, as with these other specimens of sera or corpuscles may be grouped. While it is an advantage to have, also, the sera and corpuscles of Groups I and IV, yet these are not absolutely essential, as will be seen from the following considerations. The serum of Group II agglutinates the corpuscles of Groups III and IV, while that of Group III agglutinates the corpuscles of Groups II and IV. As the corpuscles of Group I are non-agglutinable, all the groups are covered by testing the corpuscles of the unknown blood against the sera of Groups II and III. So, too, in cases in which the sera of the unknown blood is submitted for examination, all that is necessary is to have on hand corpuscles of Groups II and III for the following reasons. Corpuscles of Group II are agglutinable by sera of Groups I and III while those of Group III are agglutinable by sera

¹ Jour. A. M. A., 1916, LXVII, 808; Journal-Lancet, 1917, XXXVII, 698; Jour. A. M. A., 1918, LXX, 1221.

² Karsner states this incidence, in terms of the Jansky classification, as Group I, 42.84 per cent.; Group II, 41.38 per cent.; Group III, 10.36 per cent.; and Group IV, 5.42 per cent. (Jour. Am. Med. Assoc., 1921, LXXVI, 88; Ibid., 260). See, also, Culpepper and Ableson, Jour. Lab. & Clin. Med., 1921, VI, 276.

of Groups I and II. As the sera of Group IV will not agglutinate the corpuscles of any of the other groups, all the four groups may be detected by such methods.

Several methods have been introduced for grouping the bloods of prospective recipients and donors, the microscopic methods being the ones of choice. The test of Moss consists simply of mixing one drop of serum with one drop of an approximately 1 per cent. suspension of twice washed red cells on a cover glass and suspending over a hanging-drop slide, studying the progress of agglutination under the microscope. Minot has found that the washing of the cells, as advocated by Moss as well as by Rous and Turner in their work, is not necessary if one collects the blood in citrate solution. He, therefore, recommends the following method, which is followed in the writer's laboratory for such work. Collect 1 drop of the blood, from a prick of the finger or ear, in 1 c.c. of 1.5 per cent. sodium citrate solution in 0.9 per cent. salt solution. Then follow the Moss technic of mixing one drop of the unknown serum with one drop of this suspension of blood cells of both Groups II and III. Allow to stand for fifteen minutes at room temperature and examine under the microscope. It is unnecessary to use the hanging-drop, as the simple placing of the inverted cover glass on the ordinary glass slide will give entirely satisfactory results, especially if a film of petrolatum be placed around the edges of the slip. Brem has called attention to the fact that, in using the microscopic method, one must be on his guard lest the presence of isohemolysins may interfere with these group determinations, owing to the possibility that these may break up the agglutinated corpuscles so rapidly that the agglutination may be masked. He uses two loopful of serum mixed with 1 loopful of corpuscle suspension, instead of the drops as advocated by Minot. If one has on hand corpuscle suspensions of Groups II and III, instead of the sera of these groups, the method is the same, except that the serum becomes then the unknown element in the test.

Sanford has introduced a further modification of this method by advocating the use of dried sera of Groups II and III, against which the corpuscular suspensions of the unknown blood are tested. After determining the grouping of the blood of various individuals, two loopful of the serum of Group II and, also, of Group III, are placed on separate cover slips and allowed to dry in the air. This method has the advantage that any number of such dried specimens may be prepared and saved for future work. As the isoagglutinins are thermostabile, no effect is manifest in the later tests. On receipt of the specimen of unknown blood, a corpuscular suspension is prepared as advocated by Minot, and 1 loopful of this placed on the dried serum. This is inverted over a glass slide, rimmed on the edges with petrolatum and studied microscopically. If the unknown corpuscular suspension is to be tested the results are as follows: agglutination on both slides of Groups II and III, indicates Group IV; no agglutination, Group I; agglutination of unknown by III serum, Group II; agglutination of unknown by II serum, Group III. If unknown serum is to be tested, add one or two loopful of corpuscle suspension of known Groups II and III and proceed as above.

The results are as follows: agglutination of corpuscles on both slides, Group I; no agglutination, Group IV; agglutination of Group II corpuscles, Group III; agglutination of Group III corpuscles, Group II.¹

Von Dungern and Hirschfeld,² accepting the hypothesis of Landsteiner that the blood group depends on the presence of two agglutinins *a* and *b* and two agglutinogens *A* and *B*, have shown that the susceptible substance is present in both parents, and occurs in most of the children; while, when a particular substance (*A* or *B*) is present in only one parent, some of the children inherit it; when neither parent has a particular one of these substances, no child ever shows it. In other words *A* as an inherited character is dominant over the character not *A*, while *B* is dominant over not *B*, and the two pairs of unit characters, *A* and not *A*, *B* and not *B*, are inherited independently of each other. Ottenberg had previously noticed that the groupings were hereditary and followed Mendel's law. He states that "Medicolegally, then, if *A* or *B* is present in a child's blood, one of the alleged parents must possess it." If the blood of a child and the alleged parents have been tested, his conclusions are as follows: "If the child's blood is the correct group for the alleged parents, then we can say that the child *could* be their offspring, not that it of necessity must be. But, on the other hand, if the child's group is wrong for the two asserted parents, then one can say with absolute certainty that the child must have a parent other than one of those asserted." The value of these deductions may be very great should they prove to be infallible. Buchanan holds that these ideas are erroneous. He states "The only instances in which it appears that the blood group could be held as direct evidence would be in a family of four or more children of whom one was of a different group than the evident group represented in both parents and all four grandparents. It should be kept clearly in mind that a grandparent might be a heterozygote in virtue of which she might transmit a character to a son or daughter, who in turn might

¹ See Landsteiner, Wien. klin. Wchnschr., 1901, XIV, 1132; Descatello and Sturli, Münch. med. Wchnschr., 1902, XLIX, 1090; Hektoen, Jour. Infect. Dis., 1907, IV, 297; Jansky, Sborn. klin. y. Praze, 1907, VIII, 85; von Dungern and Hirschfeld, Münch. med. Wchnschr., 1910, LVII, 741; Ztschr. f. Immunitätsf., 1910, VIII, 520; Moss, Bull. Johns Hopk. Hosp., 1910, XXI, 63; Am. Jour. Med. Sc., 1914, CXLVII, 698; Ottenberg, Jour. Exper. Med., 1911, XIII, 425; Weil, Jour. A. M. A., 1915, LXIV, 425; Ottenberg and Libman, Am. Jour. Med. Sc., 1915, CL, 36; Lewisohn, Ibid., 886; Simons, Jour. A. M. A., 1915, LXV, 1339; Satterlee and Hooker, Ibid., 1916, LXVI, 618; Lindeman, Ibid., 624; Cherry and Langrock, Ibid., 626; Brem, Ibid., LXVII, 190; Unger, Ibid., 1917, LXIX, 2159; Williamson, Jour. Lab. and Clin. Med., 1917, II, 58; Wohl, Ibid., 516; Turner, Journal-Lancet 1917, XXXVII, 230; Meloney, Stearns, Fortune, and Ferry, Am. Jour. Med. Sc., 1917, CLIV, 733; Hedon, Presse Méd., 1917, XXV, 129; Coca, Jour. Immunol., 1918, III, 93; Lyon, Jour. A. M. A., 1919, LXXII, 1134; Drinker and Brittingham, Arch. Int. Med., 1919, XXIII, 133; Lewisohn, Presse Méd., 1919, XXVII, 593; Moffitt, Klugh and Shepard, Jour. A. M. A., 1919, LXXIII, 1821; Lyon, Jour. Am. Med. Assoc., 1920, LXXV, 1002; Zimmermann, Zentralbl. f. Gynäk., 1920, XLIV, 1146; Koeckert, Jour. Immunol., 1920, V, 529; Williams, Jour. Exp. Med., 1920, XXXII, 159; Ashby, Ibid., 1921, XXXIV, 127 and 147; Millett, Northwest Med., 1921, XXI, 68; Jones, Practitioner, 1921, CVI, 217; Unger, Jour. Am. Med. Assoc., 1921, LXXVI, 9; Belknap, Ibid., 724; Jervell, Jour. Immunol., 1921, VI, 445; Schutze, Brit. Jour. Exp. Path., 1921, II, 26; Alexander, Ibid., 1921, II, 66; Buchanan and Highley, Ibid., 247; Wearn, Warren and Ames, Arch. Int. Med., 1922, XXIX, 527; Dyke, Lancet, 1922, I, 579.

² Von Dungern and Hirschfeld, Ztschr. f. Immunität., 1910, VI, 284; Ottenberg, Jour. Am. Med. Assoc., 1921, LXXVII, 682; Buchanan, Ibid., 1922, LXXVIII, 89; Ottenberg, Ibid., 873; Buchanan, Ibid., LXXIX, 180.

be a heterozygote, and finally in the family at issue the long concealed character or group might appear." It is to be hoped that this field will be more fully investigated, as the importance of the findings in cases of disputed parentage is evident, should they prove to be established on a firm foundation.

IX. MEDICOLEGAL ASPECTS

In medicolegal examinations one is frequently called upon to determine the identity of various suspected stains. Such examinations may tax the entire resources of the worker and have, in many cases, led to absolutely negative results. It is not sufficient at the present day that the examiner state that a certain stain is blood, but he must be prepared to say what kind of blood it is. This latter point has been made possible by recent work so that one is fairly sure of limiting this statement at least to the blood of very closely associated animals.

(1) Red Cells.

It is not always a simple matter to determine that a stain on cloth, wood, iron, etc., is really due to blood. The color of the stain may be of any hue from dull red to a dirty gray, depending upon its exposure to various elemental conditions. The action of various substances, such as mortar, brick, or lime in any form, strong acids and alkalies, leather, chemicals in wall-paper, starched clothing, etc., may so change the blood or its reactivity to certain tests that no definite conclusions can be obtained.

It is self-evident that the demonstration under the microscope of blood-cells is the surest proof of the presence of blood, the size and shape of the corpuscles frequently giving a clue as to the source of the blood. It becomes, therefore, necessary to make a suspension of the stained material preferably in isotonic (0.9 per cent.) sodium chlorid solution. The degree of solubility of the stain will depend upon the age of the stain, the heat to which it has been subjected, the amount of sunlight or of moisture to which it has been exposed, and the material upon which the stain is formed. Various fluids have been advanced, from time to time, as dissolving agents for the stains, many of these are active laking or hemolytic agents, especially for dried red cells, so that one sees in such preparations only the shadow of the red cell. Such laking agents are distilled water, 0.85 per cent. ammonium chlorid solution, 50 per cent. glycerin solution, and many others. Frequently a strong 30 per cent. potassium hydrate solution will enable the worker to obtain a very good idea of the presence or absence of red cells. Marx's fluid (made as follows, hydrochlorate of quinin 10 c.c. of a 1 to 1,000 solution added to 10 c.c. of 33 per cent. potassium hydrate solution, and tinted with eosin which will stain the erythrocytes a characteristic reddish hue) is a very excellent examining fluid.

As the presence of the red cells is so hard to demonstrate, search for them is frequently omitted and reliance placed more upon the demonstration of the hemoglobin in such stains. If the stain is upon a hard surface it may be scraped off with a clean piece of glass. If it be upon cloth, a portion of the

stained part and also of the unstained part is removed and cut into small pieces with scissors which are absolutely clean. Such small pieces not broader than 1 mm. are very easily handled and can be readily teased out if desired.¹

(2) **Guaiaic Test.**

This test originally devised by van Deen is one of our oldest for the detection of blood coloring matter, but seems to be much more reliable in its negative phase than when positive. The principle of the test is as follows: To a watery solution of the suspected stain is added an equal portion of fresh tincture of guaiac. This tincture is best made by dissolving the guaiac resin in alcohol when needed. The addition of the guaiac tincture to the suspected solution causes a milky turbidity. On now adding ozonized oil of turpentine, peroxid of hydrogen, or oil of eucalyptus, so that these latter substances float upon the guaiac and blood solution, a distinct blue color will be manifested at the point of contact, and, on shaking the tube, the coloration will spread throughout the mixture. This test seems to be very delicate, demonstrating the presence of blood in a dilution of several thousand. The blue coloration observed in this test is due to the oxidation of the guaiac to guaiaconic acid, which is then further oxidized into guaiac blue by the catalytic action of the oil of turpentine. It has been shown by Taylor that this reaction is also given by many substances among which we find manganate and permanganate of potassium, peroxid of manganese, peroxid of lead, chlorin, bromin, iodine, nitric and chromic acids, ferric chlorid, salts of copper, ferro- and ferricyanid of potassium, gum acacia, gluten, unboiled milk, raw potato pulp, pus, and any living cell or its intracellular enzymes. Various enzymes such as the oxidases will give this reaction. Buckmaster advises the use of the pure guaiaconic acid along with peroxid of hydrogen in the place of the guaiac resin and believes that the test increases in delicacy and accuracy under these conditions. He states that fluids containing hemoglobin or most of its derivatives give this test when it is impossible to detect pigments by any other methods and rightly adds that boiling the fluid suspected of containing blood does not interfere with the reaction but, on the contrary, throws out of consideration the action of milk, pus, fibrin, or of any enzyme. For the proper performance of this test the fluid to be tested, should not be alkaline and only very slightly, preferably not at all, acid.

(3) **Schaer's Test.**

This test is similar in many ways to the van Deen test, but employs, instead of guaiac, a 1 to 4 per cent. solution of aloin in alcohol. On adding this tincture to the suspected solution a red color soon becoming distinctly cherry-red appears upon the addition of ozonized oil of turpentine. Many other substances give

¹ Blood stains on cloth may be removed, even after years standing by treating the stains with a drop of acetic acid and then soaking for several hours in a concentrated (70 per cent.) aqueous solution of chloral hydrate. Also, treatment with hydrogen peroxid solution for several hours will remove the stain. While these methods are reliable for the removal of blood stains, they are not to be used for forensic purposes as the tests to be applied for recognition of blood are interfered with to a great extent, although Boas employs the above chloral hydrate solution in his improved test for occult blood in feces (see Berl., klin. Wehnschr., 1916, LIII, 1357).

a pink color, but only after the lapse of one or two hours,* while the color with blood appears in a very short time. The tincture of aloin should always be freshly prepared, as of itself it undergoes this color change after standing.

(4) Phenolphthalin Test.

This test,¹ originally employed by Meyer² and, later, by Utz,³ has been so improved by Kastle and Amoss⁴ and Deléarde and Benoit⁵ that it is, probably, our most delicate one for the detection of blood, especially in stains, its limit of delicacy being about 1 part of blood in 8 million of water. As in the previous tests, the hemoglobin acts as an oxygen carrier, the active oxidizing agents being ozonized turpentine or hydrogen peroxid. The oxidases, peroxi-

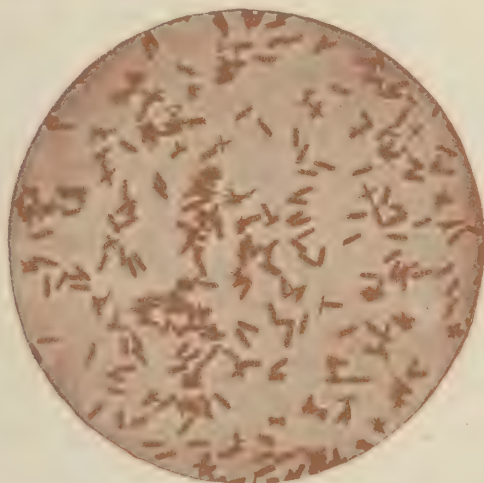


FIG. 159.—Hæmin Crystals from Human Blood. (Howk.)

Reproduced from a micro-photograph furnished by Prof. E. T. Reichert, of the University of Pennsylvania.

dases and catalases play small rôles in this process in the case of blood-stains, but, in the various secretions and excretions of the body, their influence is very great.

Phenolphthalin is a product of the reduction of phenolphthalein by zinc in alkaline solution. When oxidized in alkaline solution, it is converted into phenolphthalein with production of an intense red color. The reagent may be obtained in the market or, preferably, prepared as follows. Phenolphthalein is dissolved in a considerable excess of 30 per cent. sodium hydrate solution and boiled with an excess of zinc dust until a few drops of the strongly alkaline liquid no longer give a red color after neutralization with HCl and sufficient alkali to alkalinize the solution. The solution is then decanted from

¹ See Kastle, Bull. 51, Hyg. Lab. U. S. P. H. & M. H. S., 1909; Boas (Berl. klin. Wchnschr., 1916, LIII, 1357) advises the use of thymolphthalein.

² Münch. med. Wchnschr., 1903, L, 1489.

³ Chem. Ztg., 1903, XXVII, 1151.

⁴ Bull. 31, Hyg. Lab. U. S. P. H. & M. H. S., 1906.

⁵ Comp. Rend. Soc. de biol., 1908, LXIV, 990. See Kelly, Jour. Lab. and Clin. Med., 1916, I, 897.

the excess of zinc dust and the phenolphthalein is precipitated by acidifying with HCl. Collect the precipitate on a filter and purify by repeated crystallization from water and alcohol. This purification is continued until a white crystalline compound is obtained free from every trace of phenolphthalein (as shown by absence of red coloration on addition of alkali). Dry at room temperature or in the oven at 50° to 80°C., care being taken to avoid contact with metallic surfaces. This compound should be kept in tightly stoppered bottles in a dark place, as oxidation gradually occurs. Rarely do we find that the coloration is sufficient to interfere with the tests, but in the delicate forensic tests it may be necessary to repurify a product which has stood for some time.¹

The solution, as used in the test, is as follows: Mix a slight excess of phenolphthalein, prepared as above, with 1 c.c. of N/10 sodium hydrate solution and a few c.c. of redistilled (from glass) water, shake thoroughly and filter. To the filtrate add 20 c.c. of N/10 NaOH solution, 0.1 c.c. of 3 per cent. hydrogen peroxid solution and make the mixture up to 100 c.c. This solution shows no trace of pink coloration when fresh, but gradually acquires a color, which may become so intense that the reagent cannot be employed. In forensic work, use only freshly prepared solutions.

To 1 part of the aqueous solution of the stain or of the secretion or excretion to be tested add 2 parts of the reagent and allow to stand for a few minutes. In the presence of blood a pink to red color appears, the intensity depending on the amount of blood present. This reaction is retarded by the extracts of various animal tissues or various secretions of the body. For this reason we are never able to detect as small amounts of blood in the secretions as in watery solutions of pure blood, the limit of delicacy of all reactions being far less in the former than in the latter case. Boiling the solutions before applying the test removes most of the interfering factors. If the secretion be treated with a thick cream of albuminum hydrate suspension, the precipitate will carry down the blood pigment and thus concentrate it. A small amount of this precipitate, whether derived from saliva, urine, feces suspension, milk, or exudates, will show a decided red color when added to 2 c.c. of the reagent. Of course, in applying the test to the aqueous solution of the blood stain, no such treatment need be employed.

This test is especially recommended for the detection of blood in all secretions and excretions (boiling them before applying the test) and, particularly, in the examination of suspected stains for forensic purposes. All possible fallacies must be guarded against and confirmatory tests used if necessary.²

¹ For routine laboratory work this reagent may be prepared as follows: Two grams of phenolphthalein, 20 grams of sodium hydrate, 10 grams of zinc dust and 100 c.c. of distilled water are mixed and heated until the solution becomes colorless. Filter while hot and preserve in an amber bottle. This solution is prone to show a slight reddish tinge from time to time owing to reoxidation, so that it may be necessary to heat it with zinc dust again and refilter.

² Vas (Deutsch. med. Wchnschr., 1912, XXXVIII, 1412) calls attention to the possibility of the presence of phenolphthalein as a purgative (if feces be tested). The addition of alkali alone will give a red color under this condition. See Cade and Mulsaut, Lyon Méd., 1912, CXIX, 885; also de Jager, Berl. klin. Wchnschr., 1914, LI, 795; Schirokauer, Deutsch. med. Wchnschr., 1914, XL, 1472 and 1617; Schneider and von Teubern, Ibid., 1673; Boas, Ibid., 1915, XLI, 549.

(5) Teichmann's Test.

This is one of the most important tests for the presence of blood and when positive is conclusive proof of the kind of stain with which one is working. A drop of blood or a portion of the suspected stain is spread upon a glass slide and covered with a drop of a very dilute solution (0.01 per cent.) of common salt.¹ The salt solution is then evaporated at a low temperature. A few drops of glacial acetic acid are then placed upon the salted stain and the preparation is covered with a cover-glass. The acid is now slowly evaporated by holding the slide over a flame in such a way that the fluid steams but does not boil. As the acid evaporates, more is allowed to run under the cover-glass, this addition and evaporation being done thrice. The specimen is cooled and mounted in glycerin or distilled water, after which it may be examined with the dry lens. A successful specimen, which is not always obtained, will show the presence of numerous brownish rhomboid crystals, which are separate or grouped in sheafs or rosettes. These are the *hemin crystals* or hydrochlorate of hematin, called, after their discoverer, *Teichmann's crystals*.

It very frequently happens that the specimens are not successful, so that several slides should be made before a negative report is given. Reasons for failure may be found in the excessive heat applied to evaporate the acid, in the fact that the salt solution may have been too concentrated, or that the stain may have been very old. The addition of salt seems to be unnecessary except in the case of old blood stains or when the blood is poor in salts. It should even then be added in only very small amounts. As pointed out by Rose, when blood is mixed with iron rust the hemin test is usually negative.²

(6) Spectroscopic Examination of Blood.

Certain characteristic appearances of the blood are noted on spectroscopic examination.³ The spectrum of the various blood pigments has been given in previous sections so that only a few additional remarks are necessary in this place. In spectroscopic examinations, the use of the ordinary hand spectroscope is all that is necessary, the more complicated ones adding little to the differentiation of the various spectra. If the blood be fresh and the hemoglobin unaltered the spectrum is, of course, that of oxyhemoglobin; but as the usual stains submitted for examination are frequently very old ones, such a spectrum is practically never seen. The end-product of the alteration of hemoglobin as found in old blood stains is hematoporphyrin, which is iron-free and can be identified easiest by the spectroscope. The suspected stain is dissolved in concentrated sulphuric acid yielding a reddish-violet fluid, which is then examined spectroscopically. The spectrum of hematoporphyrin has been given previously. Many blood stains can be recognized only by this

¹ Nippe (Deutsch. med. Wchnschr., 1912, XXXVIII, 2222) advises one to mix the specimen with a few drops of a solution of 0.1 gram each of potassium bromid, iodid and chlorid in 100 grams of glacial acetic acid. This modification shows up the crystals even when blood is mixed with rust. See, also, Symons, Biochem. Jour., 1913, VII, 596; Strassmann, Münch. med. Wchnschr., 1920, LXVII, 748.

² See Symons, Biochem. Jour., 1913, VII, 596; Beam and Freak, Ibid., 1915, IX, 161; Bokarius, Vierteljahrschr. f. gerichtl. Med., 1918, LV, 255.

³ Lewis reports a study of the ultraviolet absorption spectra of blood sera (Proc. Roy. Soc. London, 1916, LXXXIX, 327).

test, so that it is always advisable to make a spectroscopic examination, especially if other tests for hemoglobin have failed.¹

(7) Precipitin Test.

The precipitin test has been discussed in great detail previously (page 718). This is the absolute identification test for a stain, providing the stain has been shown to be a blood stain by the tests given above. The precipitin test is specific, within very narrow limits, and is recognized in law as reliable in differentiating human blood from that of the domestic animals.² Its one fallacy is that it reacts with the blood of anthropoid apes just as it does with that of man, but in most cases this element may be easily excluded.

X. VALUE AND LIMITATIONS OF BLOOD EXAMINATIONS

It has been truly said that the value of a blood examination "is measured by the practical use which may be made of it, and not by any interesting yet useless information it may throw on the case." In clinical work we are prone to follow well-established lines of procedure and to forget, when engaged in counting the cells in an obscure case, that "the meaning and aim of the clinical study of the blood covers a much wider field than is embraced in the mere investigation of the histological elements." Yet our knowledge regarding the variations in the plasma is so meager that the busy practitioner may be pardoned for such neglect.³

There can be little question that the so-called "blood diseases" have the most characteristic variation from the normal, yet even here it is not so easy, as one might assume, to make an indisputable diagnosis in all cases. Thus, aside from the pure parasitic diseases, leukemia and advanced pernicious anemia seem to be the only ones capable of a certain diagnosis from the examination of the blood. True the various anemias may be detected, but the etiology is not always clear nor the differentiation of a primary from a secondary form invariably possible.

In recent years the study of the chemistry of the blood has assumed great importance. There can be little question that carefully controlled chemical examinations of the blood do give information which can be obtained in no other way. The importance of a determination of the amount of the various nitrogenous constituents and the relation of these amounts to those excreted in the urine is self evident when attempts are made to make careful diagnoses and prognoses of conditions associated with an acidosis. While these determinations require a certain experience in chemical technic, yet the different methods advised are soon mastered by close study, so that one may avail himself of the benefits offered by routine use of such methods in properly selected cases. Further, a study of the blood sugar will, often-times, make a differential diagnosis between a true diabetes from one of the

¹ See Sutherland and Mitra, *Biochem. Jour.*, 1914, VIII, 128; Heller, *Vierteljahrsschr. f. ger. Med.*, 1916, LI, 219 calls attention, to the importance of fluorescence of hemoglobin derivatives in forensic blood tests.

² See Ekeley, *Jour. Lab. & Clin. Med.*, 1921, VI, 709.

³ See Butterfield and Stillman, *Am. Jour. Med. Sc.*, 1917, CLIV, 783.

"renal" type as well as from a glycosuria, induced by purely alimentary excess of carbohydrate. A study of these methods is strongly recommended.

It is, of course, evident that bacteriologic examination of the blood and the application of the agglutination reaction may clear up many an obscure case, but such work requires much more time and much more attention to detail than is at the disposal of the busy practitioner. It is, however, in just this class of infectious fevers that much benefit, in a confirmatory way, is forthcoming from a careful study of the blood. Thus, a leucocytosis or a leucopenia in any suspected case might lead to a differentiation of central pneumonia from typhoid fever or a scarlet fever from a case of measles. Moreover, a leucocytosis following a leucopenia in typhoid would strongly indicate perforation and consequent surgical interference. Suppurative processes anywhere in the system are usually associated, unless very limited in extent, with a polynuclear neutrophile leucocytosis; so that the leucocyte count may be of great importance in judging of the extension of a pus infection. Immediate operation is occasionally decided upon after a leucocyte count, but this should not be made the only basis for such intervention unless frequent counts have given the surgeon the definite knowledge that a leucocytosis has suddenly occurred.

It should be stated as an axiom that blood findings may never be interpreted except in the light of the clinical findings. To the clinician it should be said, never trust the laboratory report implicitly unless it agrees with the clinical manifestations; to the laboratory worker we would say, never report a blood finding as diagnostic without knowing something of the clinical history of the case.

A single blood examination will rarely be of any greater value than will a single temperature determination. It is the series that enables one to decide either as to diagnosis or as to operative or therapeutic procedures. What we need most is not so much blood work, but much better work when done. Too much reliance is often placed on blood reports, so that a word of caution does not seem ill advised. Never rely solely on the blood findings, but use them merely as one of many links in your chain of clinical evidence. In this way mistaken diagnoses will occur less frequently.

CHAPTER IX

TRANSUDATES AND EXUDATES

I. GENERAL CONSIDERATIONS

The serous membranes are normally kept moistened by liquids whose quantity is only sufficient in a few instances, as in the pericardial cavity and the subarachnoidal space, for a complete chemical analysis to be made of them. Under pathological conditions an abundant transudation may take place from the blood into the serous cavities, into the subcutaneous tissues or under the epidermis. If such conditions be the result of circulatory disturbance, the kidneys are usually unable to eliminate the normal amount of fluid from the system and, as a result, the retained fluid collects both in the serous cavities and in the areolar tissue. Such accumulations of fluid, known as *transudates*, are similar to the lymph, being, as a rule, poor in cellular elements and yielding little or no fibrin. These transudates must be sharply differentiated from the accumulations of fluid, which are the result of direct inflammatory processes in the membranes lining the serous cavities and are known as *exudates*. These latter fluids are generally rich in cellular elements and yield relatively more fibrin. As a rule, the richer a transudation is in leucocytes, the closer it stands to pus, while the poorer it is in these elements the closer it resembles true lymph.

The formation of true transudates is largely a question of filtration under the influence of the rate of blood flow, the blood pressure, the irritation of the capillary endothelium, and the variable permeability of the endothelial cells. We should expect, therefore, that the passage of dissolved substances from the blood would be regulated by the same laws that control the secretion of physiologic fluids, namely the laws of passage of fluids through semipermeable membranes. The crystalloids would be, therefore, in approximately the same concentration as in the blood plasma, while the colloids must be in far less concentration, the actual values being influenced, of course, by the special membrane through which the fluid passes. The condition of the blood would hence affect the chemical composition of such transudates, hydremic plasma yielding a fluid poorer in solids, while anhydremic blood is associated with a transudate of higher specific gravity.

From a clinical standpoint a differentiation between transudates and exudates is not infrequently impossible, so that it is advisable to resort to aspiration of the fluid and to chemical and microscopical examination of the material withdrawn. The chief phases of such examinations are: (1) the chemical and physical properties of the fluid; (2) the bacteriological aspect of the fluids, and (3) the morphological characteristics of the cellular elements.

Obtaining the Specimen.

Whenever fluid is to be withdrawn, either for diagnostic or therapeutic purposes, it is necessary to resort to puncture of the cavity containing the fluid. In all cases the site of puncture must be as carefully prepared as in any surgical procedure. Puncture may be performed with the ordinary trocar or, preferably, with a large needle which has a rather large lumen. The instruments must be carefully sterilized before use. If the skin is especially tough, it is advisable to make a small incision through the skin and insert the needle through the incision. Very little pain is felt by the patient during this procedure, but the writer is accustomed invariably to resort to the use of ethyl chlorid to anesthetize the part.

In the withdrawal of pleuritic effusions the spot selected should be neither too high nor too low. It may be in the seventh intercostal space in the axillary line or in the eighth intercostal space at the outer angle of the scapula. The arm of the patient should be brought forward with the hand resting on the opposite shoulder, in order to widen the intercostal spaces. In inserting the needle it is wise to make the thrust close to the upper margin of the rib so as to avoid the intercostal artery. In all cases the fluid should be withdrawn slowly and the excess above that required for examination allowed to drain until the desired amount is obtained. If the puncture is for diagnostic purposes, 10 to 20 c.c. are sufficient, while the therapeutic withdrawal of fluid will vary with the amount present and with the clinical symptoms of the case. Should the patient show signs of shock or of depression during the operation, the procedure should be interrupted as quickly as possible. Aspiration is rarely necessary or advisable.

In the withdrawal of fluids from the abdominal cavity, the needle or trocar is thrust through the lower abdominal wall and the fluid collected. In this procedure more or less danger of puncturing the bowel exists if the effusion be small, so that the needle should not be carelessly inserted lest this complication arise. Naturally, in well-marked ascites no such danger is present.

II. PHYSICAL AND CHEMICAL PROPERTIES

Transudates are, as a rule, serous in character, usually transparent, colorless, or light yellow in color, but at times showing a milky, reddish, or a greenish tinge, the latter practically always being observed after the fluid has stood exposed to the air. Such solutions are, as a rule, dichroic, yellow by transmitted light and green by reflected light. They are alkaline in reaction and show a specific gravity, which varies, according to the origin of the fluid, from 1,006 to 1,018, while serous exudates from the same cavities show a much higher specific gravity.¹ The variations in specific gravity depend largely upon the amount of albumin present in the transudate, this practically never being over 3 per cent. and usually much lower. The chief proteins present are albumin and globulin, these being related to one another in the transudates as one and one-half to one, while in the exudates the globulin

¹ See Trevisan, *Ztschr. f. exper. Path. u. Therap.*, 1912, X, 141.

is relatively much increased.¹ The determination of the total protein may be made by methods of fractional precipitation as previously discussed. The transudates from the pleura contain the largest percentage of albumin, while edematous fluids rarely show over 1 per cent. Transudates do not coagulate spontaneously. Glucose is present both in transudates and exudates in amounts varying between 0.04 and 0.1 per cent. The mineral constituents of transudates are somewhat higher than in the exudates, the former averaging 0.06, the latter 0.89 per cent. Under pathological influences fat, blood, uric acid, and biliary pigment may find their way into both types of fluid. In diabetes an excess of sugar and the presence of acetone bodies may be detected.

The exudates are usually straw- or lemon-yellow in color depending on the degree of inflammation, or they may assume colorations ranging from a deep red (hemorrhagic) to a milky (purulent) shade. Biliary pigments may cause a bright green, while various medicaments, such as methylene blue, may produce a greenish-blue coloration. The specific gravity is almost invariably above 1.018, the reaction is alkaline, the albumin content is usually above 3 per cent., reaching as high as 7 per cent., while the globulin is relatively much increased in comparison with the albumin. This globulin increase is largely due to paraeuglobulin. Nucleoprotein is especially abundant in purulent exudates in which the autolytic processes are more or less marked. The total nitrogen of the various fluids varies from 0.22 to 1.38 per cent. The nitrogen partition may be seen from the following table of Gerhartz.² Exudates coagulate spontaneously on standing.³

Figures in terms of % (grams per 100 c.c.)	Total N	Precipitable N	Protein N	Ammonia N	Purin N	Urea N	Amino-acid N
Transudate.....	0.22-0.58	0.18-0.53	0.17-0.52	0.007-0.01	0.002-0.007	0.01-0.05	0.002-0.005
Serous exudate.....	0.43-1.09	0.39-1.07	0.37-1.03	0.01-0.03	0.01	0.01-0.06	0.007
Purulent exudate...	1.11-1.33	0.94-1.22	1.14	0.01	0.007	0.02-0.1	0.004-0.28

The exudates, which, accurately speaking, are always of inflammatory origin, may be serous, serofibrinous, seropurulent, hemorrhagic, purulent, putrid, chylous, and chylid.

Serous Exudates.

These are clear, of a light straw color, and show a specific gravity above

¹ See Mosny, Javal and Dumont, *Presse méd.*, 1914, XXII, 477; Ujihara, *Biochem. Ztschr.*, 1914, LXI, 55; Berl. klin. Wehnschr., 1914, LI, 1112; Villaret, *Jour. de Physiol. et de Path. gen.*, 1913, XV, 617, 652 and 875; Natali, *Riv. Crit. di Clin.* 1921, XXII, 337 and 349.

² *Chemie der Transudate und Exsudate*, Jena, 1908. See, also, Wiener (*Biochem. Ztschr.*, 1912, XLI, 149) regarding the presence of proteolytic ferments and amino-acids in exudates; and Lenk and Pollak, *Deutsch. Arch. f. klin. Med.*, 1913, CIX, 351. See Hegler and Schumm, *Med. Klin.*, 1913, IX, 1810; Dorner, *Deutsch. Arch. f. klin. Med.*, 1914, CXIII, 342; Denis and Minot, *Arch. Int. Med.*, 1917, XX, 879; Schulman, *Jour. A. M. A.*, 1917, LXVIII, 1256, reports a case of pleural effusion containing a large percentage (0.73) of cholesterol.

³ Rivalta advocates the following test to differentiate an exudate from a transudate: In a conical glass place about 200 c.c. of water and add 2 drops of glacial acetic acid. Mix thoroughly and allow 1 or 2 drops of the puncture-fluid to fall into this weak acid solution. If the fluid be an exudate a distinct cloud will be observed in the wake of the falling drop, while if it be a transudate little or no turbidity will be noticed. This reaction is due to the large globulin content of the exudate. See, also, Delrez, *Bull. Acad. Roy. Méd. Belg.*, 1919, XXIX, 733; Larsen and Secher, *Hospitalstid.*, 1921, LXIII, 273.

1,018. There is a large amount of fibrin, as shown by the dense network microscopically, containing a few red cells which may be derived from the bleeding at the point of puncture, a few leucocytes which may vary in type according to the kind of bacteria causing the infection, and large endothelial cells from the serous membrane lining the cavity. If the blood-cells be present in sufficient numbers to give a distinct red color to the fluid it is termed a hemorrhagic exudate, while if a few pus-cells are found it may be called a seropurulent type. The gradations between the true serous and serofibrinous types of exudate are exceedingly varied, so that it is difficult to tell which type is really present. Even from a purely serous exudate a certain amount of coagulation, with formation of a distinct fibrin network, may be obtained, so that the only criterion would be one of degree.¹

The type of leucocyte present is usually of the polymorphonuclear variety, although other forms may be present. It is, therefore, an important part of the examination of an exudate to determine the percentage relations of these various cellular elements. This will be discussed in the section on Cytology.

Chylous Exudates.

On account of the close relationship between the abdominal, thoracic, and pericardial cavities, on the one hand, and the large lymphatic trunks, on the other, it is possible for lymph to pass directly into these cavities in case rupture of these vessels occurs. Such exudates show all the properties of chyle. The fluid is white and milky, contains between 1.5 and 2.5 per cent. of protein, and a considerable amount of fat, which may be demonstrated by staining with osmic acid or Sudan-III or by alkalinizing with sodium hydrate and shaking out with ether.

Chyloid Exudates.

It has been found that carcinoma, tuberculosis, extreme cardiovascular changes, hepatic disturbances, puerperal sepsis, and infection with the filaria may give rise to a chyloid type of ascites. The fluid in these cases is less milky than that of the true chylous type, contains less fat, and is not so completely cleared by shaking with ether. It contains serum-albumin, mucin and, especially, rather large amounts of a complex of pseudoglobulin and lecithin, to which the opacity is due.² Traces of sugar are found but not as large in amount as in the true chylous types. These fluids resist putrefaction for long periods owing to their lecithin content. The accumulation of fluid after tapping is much more rapid in the chylous than in the pseudo-chylous types.

Hemorrhagic Exudates.

This type is, in reality, a serofibrinous form containing large numbers of blood-cells. It is observed in patients with hemorrhagic diathesis, in connection with active tuberculosis, with neoplasms of the serous cavities, and following injuries to the chest or abdomen. In this form of exudate, the

¹ See Epstein, *Jour. Exper. Med.*, 1914, XX, 334.

² See Wallis and Schöberg, *Quart. Jour. Med.*, 1910, III, 301; *Ibid.*, 1911, IV, 153; also Henry, *New York Med. Jour.*, 1911, XCIV, 1; and Leclard, *Brit. Jour. Child. Dis.*, 1913, X, 433; Fubini, *Gazz. d. osp.*, 1915, XXXVI, 145; Outland and Clendening, *Jour. A. M. A.*, 1916, LXVI, 1833; Lewin, *Am. Jour. Med. Sc.*, 1916, CLII, 71; Brenans, *Jour. pharm. chim.*, 1920, XXI, 228; Welker and Williamson, *Science*, 1920, LI, 397; Guldman, *Hospitalstid.*, 1921, LXIV, 113.

exciting bacterial agent, usually the tubercle bacillus, may occasionally be found, but not always. The type of leucocyte (mononuclear) would be very strong presumptive evidence in favor of tuberculosis, even though no bacilli were found. If the exudate be due to a malignant growth, it is sometimes possible to obtain shreds of the tumor tissue and thus make a probable diagnosis. In judging of the malignancy of the cells, it is sometimes difficult to differentiate the abnormal from the normal, especially in the affections of the pleura. These malignant cells are usually extremely large and are characterized by their vacuolation and fatty degeneration (see cut). It is not infrequent to find in hemorrhagic exudates, which have remained in the body cavity for some time, a large number of cholesterin crystals and occasionally small masses of hemosiderin.

Purulent Exudates.

These are composed either of true pus or of seropus. They are more or less yellow in color, thick and occasionally tenacious, and separate on standing or centrifuging into a cellular deposit and a pus serum. The cells forming the pus are not infrequently in a condition of advanced fatty degeneration and may contain numerous bacteria. The addition of acetic acid will usually clear up the cells so that the nuclei become recognizable. Fat is present in amounts as high as 7 per cent., while a relatively large amount of various proteins and extractives is shown on chemical examination. Fatty acid crystals and cholesterin may be abundant in the sediment, especially in old abscesses. Fresh pus is usually alkaline, but may become distinctly acid, owing to the development of lactic acid in the process of autolysis.¹

Purulent exudates are investigated with special regard to the type of cell present and to the organism associated with the pus formation. In ordinary pus, the cell is of the polymorphonuclear type, although occasionally the mononuclear form may be predominant. The bacteria are very numerous and include many of the most important types. While the pyogenic bacteria are more frequently the various types of staphylococci and streptococci, it is to be remembered that other organisms may produce pus under certain conditions. Thus the typhoid bacilli, colon bacilli, pneumococci, Friedländer's bacilli, gonococci, diphtheria bacilli, Morax-Axenfeld and Koch-Weeks bacilli, and influenza bacilli, among others, may be the causative factor in the formation of a purulent exudate. It will be seen, therefore, that the bacterial examination of a purulent exudate may require a very extensive research. As it is frequently impossible to decide, from a stained specimen, as to the special organism causing the infection, cultures should be made in every doubtful case. The peculiarities of such cultures may be found in any work on bacteriology.

Putrid Exudates.

These may be observed in various cavities of the body or in the substance of various organs, especially the liver and lungs. They arise from the entrance of pus into these cavities from perforation of a gangrenous area, gastric or intestinal ulcer, malignant growths, etc. The material obtained by puncture is usually brownish or greenish in color, has a very offensive odor,

¹ See Ito, Jour. Biol. Chem., 1916, XXVI, 173.

and is usually alkaline, but may be acid. Microscopically degenerated cells, numerous bacteria, cholesterin, fatty acid, and hematoidin crystals are observed. In some cases various portions of an echinococcus cyst may be found in the exudates. Bilirubin crystals and various amino-acids may be found in rupture of an hepatic abscess.

III. BACTERIOLOGY

It is always advisable to make cultures of the material obtained by puncture in order to discover the organism which is acting as the exciting cause of the condition under investigation. Usually a few c.c. of the fluid are allowed to drop into a flask containing 50 c.c. of sterile nutrient bouillon and the mixture incubated for 24 to 48 hours. From the growth, obtained in this preliminary work, subcultures are made on various media and microscopical examination employed to identify the organism. It is always advisable to make at least two microscopical specimens, staining one with the ordinary methylene blue stain and the second with Gram's stain.

Tubercle Bacilli.

If these organisms are suspected, the technic of staining is the same as outlined under Sputum. It frequently happens, in the examination of suspected tubercular exudates, that the presence of a large amount of fibrin, either with or without spontaneous coagulation of the specimen, makes it exceedingly difficult to find the organism, even though it be present. In such cases, advantage is taken of a procedure, recommended by Jousset,¹ known as *inoscopy*. If the fluid has not coagulated, it is allowed to do so in order that the coagulation may enclose the bacilli within the fibrinous network. The coagulum is separated as completely as possible from the supernatant fluid, is washed with distilled water, and is treated with 30 to 40 c.c. of the following mixture, which will digest the fibrin.

Pepsin,	2 grams.
Sodium fluorid,	3 grams.
Glycerin,	10 c.c.
Hydrochloric acid (conc.),	10 c.c.
Distilled water, q.s., ad.,	1000 c.c.

This mixture is placed in the incubator for 24 hours, when the fluid becomes homogeneous. The digested fluid is centrifuged and smears are made from the sediment as previously described. It is always advisable to treat such smears with a small quantity of albumin fixative, so that the organisms may not be washed from the slide. The smear is fixed in the flame and stained in the usual way with carbol fuchsin. The tubercle bacilli may not appear as deeply stained after such treatment. This method has been replaced, to a large extent, by the Antiformin method (see p. 18).

Gonococcus.

The gonococcus appears in stained specimens as small biscuit-shaped or coffee-berry-shaped cocci, which are arranged in pairs separated by a narrow

¹ Semaine Méd., 1903, XXIII, 22. See Cheer, Jour. Am. Med. Assoc., 1922, LXXVIII, 1612.

unstained portion. Occasionally two of these pairs of hemispheres are joined together, forming tetrads. They may be stained by any of the anilin dyes, but are recognized especially by their reaction toward Gram's method. In this connection it must be said that other organisms, among which we find the diplococcus intracellularis meningitidis of Weichselbaum and the micrococcus catarrhalis, resemble the gonococcus both in morphological and staining characteristics, so that a differentiation by this method is not always possible. Fortunately, however, the cultural peculiarities of these organisms absolutely differentiate them.¹

Gram's Stain.

The principle of the staining of various organisms by Gram's method is that certain organisms retain the primary color after treatment with decolorizing agents, while others lose this primary stain and must be treated with a contrast stain for their recognition. If the organism retains the primary blue color it is called Gram-positive, while if it lose the primary color and take on the contrast stain it is called Gram-negative. Among the organisms which are Gram-negative we find the gonococcus, meningococcus, micrococcus catarrhalis, influenza bacillus, typhoid bacillus, colon bacillus, Koch-Weeks bacillus, and the Morax-Axenfeld bacillus; while the tubercle bacillus, smegma bacillus, diphtheria bacillus, pneumococcus, streptococcus, staphylococcus, and various saprophytic cocci found in the smears both of the male and female urethra are Gram-positive.

The solutions required in Gram's method are: (1) an anilin oil—gentian violet mixture, consisting of 84 c.c. of anilin water (water saturated with anilin and filtered), and 16 c.c. of a saturated alcoholic solution of gentian violet; (2) a solution of iodine consisting of one gram of iodine, 2 grams of potassium iodid, dissolved in 300 c.c. of water; (3) a dilute solution of carbol fuchsin, a dilute aqueous solution of safranin, or a 1 per cent. aqueous solution of Bismarck brown as a contrast stain. Jensen² has shown that 0.5 per cent. aqueous solution of methyl violet (6B) may be used as the primary stain and, thus, obviate the necessity of preparing fresh anilin water solutions, as applied in the original Gram method. This worker advocates, also, the use of a somewhat stronger iodine solution, namely, iodine 1 gram, potassium iodid, 2 grams, dissolved in 100 c.c. of water.

¹ See Warden (Jour. Infect. Dis., 1913, XII, 93), who emphasizes the importance of a slightly acid reaction in the media containing preferably human body fluids, such as ascitic or hydrocele fluid. Warden (Jour. Infect. Dis., 1913, XIII, 124) calls attention to the fact that, oftentimes, what appears to be the gonococcus in smear preparations is shown to be a staphylococcus on culture. This awaits confirmation. See, also, Whitney, Boston Med. and Surg. Jour., 1914, CLXX, 749; France, New York Med. Jour., 1914, C, 1255; Wolbarst, Ibid., 1915, CI, 146; Warden, Jour. Infect. Dis., 1915, XVI, 426; Pearce, Jour. Exp. Med., 1915, XXI, 289; Eastman, Ill. Med. Jour., 1916, XXX, 396; Culver, Jour. A. M. A., 1916, LXVI, 553; Ibid., 1917, LXVIII, 362; Warden, Ibid., 432; Herbst, Ibid., 761.

² Berl. klin. Wehnschr., 1912, XLIX, 1663; see, also, Special Report No. 19, Med. Res. Comm., National Health Insurance, London, 1918, p. 6; Stovall and Nichols, Jour. A. M. A., 1916, LXVI, 1620. Burke (Jour. Am. Med. Assoc., 1921, LXXVII, 1020; Jour. Bact., 1922, VII, 150) recommends the mixing with the stain on the slide 3 to 8 drops of 5 per cent. solution of sodium bicarbonate in order to increase the penetration of the dye and the holding power of the Gram-positive organisms for the primary stain. He uses acetone as the decolorizing agent. See, also, Benians, Jour. Path. and Bact., 1920, XXIII, 401; Deussen, Biochem. Ztschr., 1920, CIII, 123; Hucker, Jour. Bact., 1921, VI, 305; Israeli, Jour. Am. Med. Assoc., 1921, LXXVI, 1497; Tunncliffe, Ibid., 1922, LXXVIII, 191.

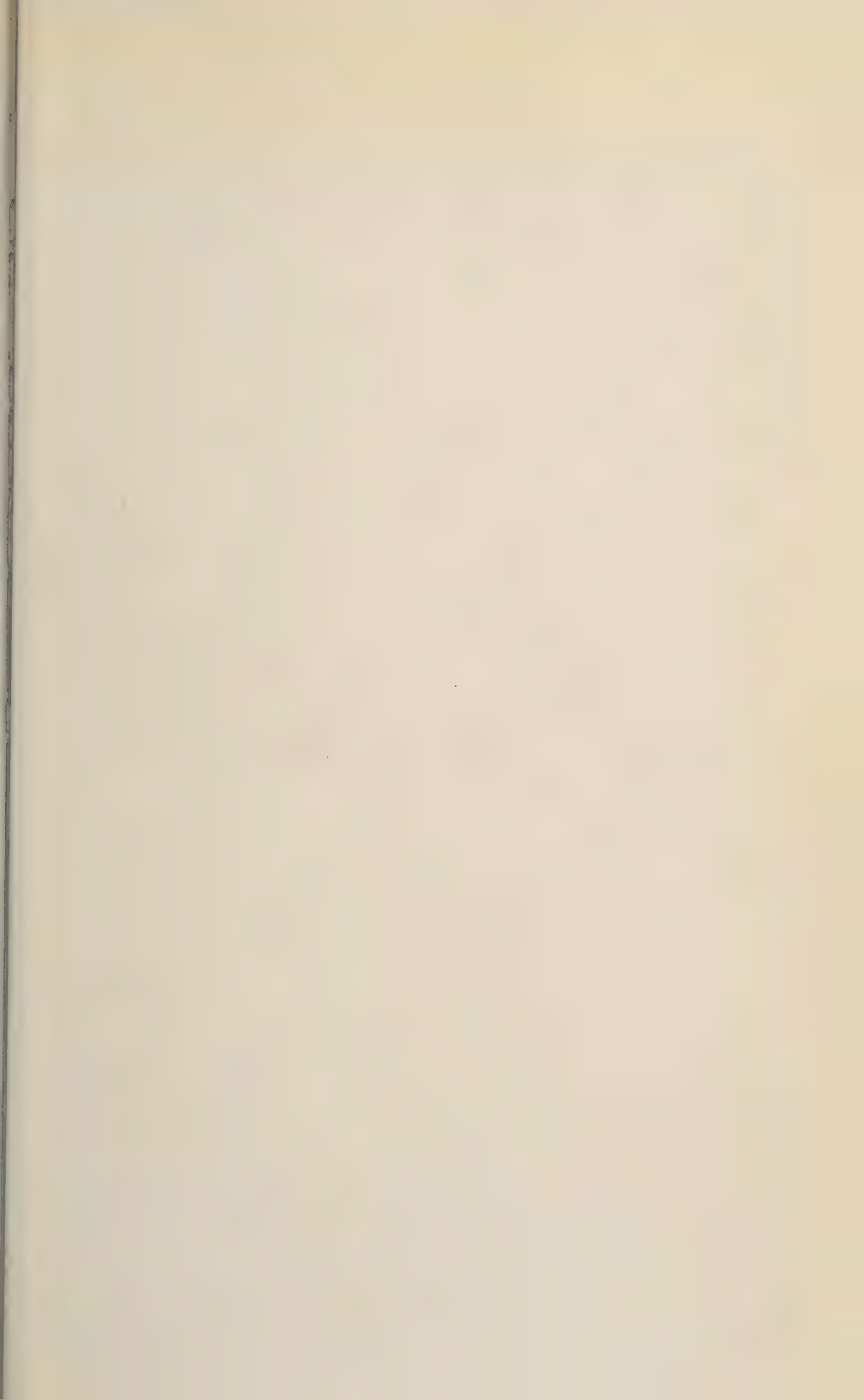
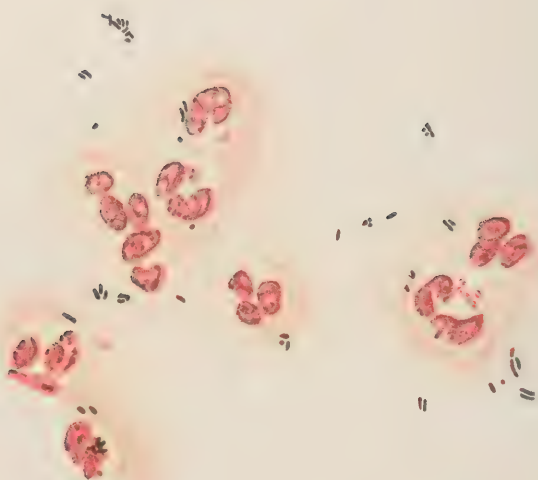


PLATE XXXIII.



Katharine Hill.

GONOCOCCI IN URETHRAL DISCHARGE. (GRAM'S STAIN.)

Technic.

Smears of the exudate are made, in the manner previously described for making blood smears, by receiving a drop of the purulent material upon one end of a glass slide and spreading it in a thin even layer by means of a second slide. These smears are then fixed by passing several times through the flame. When the slides have cooled, they are covered with the gentian-violet solution which is allowed to act for one to three minutes. The solution is then poured off and the excess removed by washing in water. Without drying, the iodine solution is placed on the slide and allowed to act for one-half to one minute. It is then washed in water and the preparation treated with 95 per cent. alcohol until no more color is removed by it.¹ The alcohol is then removed by washing with water and the smear is covered with one of the contrast stains above mentioned, in the writer's laboratory the safranin solution, which is allowed to act for only a few seconds. The stain is then washed off with water and the slide dried between folds of filter-paper.

The specimen under the microscope shows the Gram-positive organisms stained deep blue, while the Gram-negative bacteria and the bodies of the pus cells take the red safranin stain. In such specimens the gonococcus, if present, will be observed both intracellularly and extracellularly, the former being the more characteristic. The ordinary pus cocci may likewise be intracellular, but these are distinctly Gram-positive, while the gonococcus is Gram-negative.

In the purulent exudate from the urethra, large masses of mucoid material may be present, which are known as gonorrheal threads. These may be found in the urinary sediment and are usually easily recognized. They contain masses of pus cells, within which may be found numerous gonococci. These shreds may persist for years in anyone with a history of a previous gonorrhea, but they may then contain no organisms. The cytology of gonorrheal pus presents nothing characteristic beyond the presence of numerous eosinophiles with large numbers of polynuclear neutrophils and an occasional lymphocyte.

It is to be stated that the gonococci need not be intracellular, as all or almost all of them may be extracellular in the very acute stages of the disease and, also, in some cases of the chronic type. Further, it is to be remembered that some of the gram-positive organisms, when taken up by the leucocyte, may lose their staining characteristics and become gram-negative. Also, in certain chronic cases, gram-negative organisms, such as the gonococcus, may hold the primary stain so tenaciously that examination shows these organisms to be gram-positive, although cultures indicate them to be true gram-negative gonococci. Such results are, fortunately, rare, and are due to the improper preparation of the slides.²

¹ Lyon (Jour. Am. Med. Assoc., 1920, LXXV, 1017) recommends the use of acetone, kept dehydrated with calcium chlorid, for decolorizing as he finds it very efficient, cheaper and more easily obtained.

² Oliver and Wherry (Jour. Inf. Dis., 1921, XXVIII, 341) call attention to the possible presence of the *diplococcus reniformis* of Cottet, which occurs as bean-shaped diplococci slightly smaller than the gonococci. This organism has been isolated from cases of vaginitis and, hence should be remembered as a possible source of confusion. It is to be recalled that the micrococcus catarrhalis not infrequently is found in the genito-urinary tract of both female and male, so that cultural methods may have to be applied before an absolute diagnosis is possible.

Smegma Bacilli.

In the exudate of the preputial follicles, known as *smegma preputii*, are found fat globules, ammonium soaps, cholesterin crystals, and a few epithelial cells. In this smegma are found many bacilli, smegma bacilli, which show the same morphological and practically the same staining characteristics as the tubercle bacillus. This is differentiated, as described under Sputum. If the urine is to be examined for tubercle bacilli, it is much better technic to use a catheterized specimen than it is to resort to methods of questionable differentiation. If this is done any acid and alcohol-fast bacilli may be regarded as the tubercle bacilli, but in all cases the smegma bacilli must be excluded.¹

Ducrey's Bacillus.

This bacillus is the organism causing soft chancre and is known as the bacillus ulceris cancrisi. It is found in the purulent discharge from the chancre, but rarely, if ever, in pure culture.¹ The sections of tissue may, however, show none but this organism.

In making preparations for study of this organism, the ulcerated surface is scraped with a platinum loop, the pus spread upon a slide, dried in the air, and fixed with alcohol-ether or over the flame.² It stains readily with all bacterial stains, but decolorizes with Gram's stain.

In stained specimens the organism appears as a short, thick, oval bacillus with rounded ends and two lateral indentations, which occasionally give it the appearance of a figure 8. Ordinarily the extremities are more deeply stained than the central portion, this appearing almost clear. The organism has a tendency to form chains or groups, which are rarely found in the pus cells but are frequently seen within the epithelial cells.³

Spiroonema Pallidum.

The Spiroonema Pallidum, discovered by Schaudinn and Hoffmann,⁴ is now definitely established as the causative factor of syphilis. It may be obtained from the primary chancre, the incised papules, condylomata, mucous patches, inguinal glands, as well as from the internal organs in tertiary and congenital syphilis. Its size, form, type of motility and method of cultivation have been discussed under blood. Noguchi⁵ states that there are many

¹ See Brereton and Smith, Am. Jour. Med. Sc., 1914, CXLVIII, 267.

² Teague and Deibert (Jour. Urol., 1920, IV, 543; Jour. Med. Res., 1922, XLIII, 61) recommend the transference of the pus to small culture tubes containing rabbit blood clotted by heating for 5 minutes to 55°C. and incubation of this culture for 20 to 24 hours. Chain forms of small gram-negative bacilli are characteristic.

³ In 1904 Scherber and Müller (Arch. f. Dermat. u. Syph., 1904, LXXVII, 77) isolated and identified in cases of "erosive and gangrenous balanitis" (the so-called "fourth venereal disease") a fusiform bacillus and a spirochete, which appears to be similar, if not identical, with the organism of Vincent, discussed previously as the cause of certain types of stomatitis. This organism has been given the name Spiroonema (spirochete) balanitidis and is rather thick as compared to the pallida and possesses very active motility, the motion being wave-like and rotary. It is about 0.2 microns in width and from 5 to 15 microns in length. There are usually from 6 to 10 spirals and are not as regular as those of the treponema pallidum. See, Corbus and Harris, Jour. A. M. A., 1900, LII, 1474; Corbus, Ibid., 1913, LX, 1769; Noguchi, Jour. Lab. and Clin. Med., 1917, II, 383; Owen and Martin, Ibid., 862.

⁴ Arbeiten aus der kais. Gesundheitsamte, 1905, XXII, 527.

⁵ Jour. Lab. and Clin. Med., 1917, II, 365 and 472; Jour. Exper. Med., 1918, XXVII, 667; Ibid., XXVIII, 559.

varieties of spirochete found in or about the body cavities, alimentary tract and genitalia and that these are nonpathogenic. He cites 8 species of *Spiro-nema* and 5 of *Treponema* as follows: *Spiro-nema* refringens, *S. microgyratum*, *S. buccalis*, *S. acuminatum*, *S. obtusum*, *S. pseudopallidum*, *S. eugyratum*, *S. stenogyratum* and *Treponema macrodentium*, *T. medium*, *T. microdentium*, *T. dentium*, and *T. calligyrum*. Of these he shows that *Spiro-nema* refringens, *Treponema calligyrum* and *T. minutum* (formerly regarded as identical with *T. microdentium*) are constantly observed in the male and female genitalia.

To obtain this organism from the primary lesions, the chancre is thoroughly cleansed either with normal salt solution or with soap and water, rinsed with salt solution and dried. If the sore is healed, the epithelial covering is removed. The chancre is lightly curetted or the edges scraped, the blood, which should be avoided, is wiped away and the serum which exudes used for the later work. A drop of this serum is placed upon a slide, covered with a cover-glass and examined at once under the dark-field illuminator, the spiro-nema appearing as colorless, glistening, rapidly motile spirals. If this method, which is to be preferred to any staining method, is not possible, a thin smear is made from the serum, transferring a drop by means of a platinum loop to a slide and using a rapid circular motion in spreading. The specimen is dried in the air and stained as follows.¹

Giemsa's Stain.

The air-dried film is fixed with chemically pure methyl alcohol and stained with Giemsa's stain (p. 577) for 20 hours, using 1 drop of the stain to 1 c.c. of water. The spiro-nemata are stained a violet-red color, the refringens being blue.²

Ghoreyeb's Method.³

The smears are made as above and air-dried. Cover the smear with 1 per cent. aqueous solution of osmic acid for 30 seconds. Wash thoroughly in running water. Cover with solution of lead subacetate (liquor plumbi subacetatis diluted 100 times) for 10 seconds. Wash in water. Cover with 10 per cent. aqueous solution of sodium sulphid for 10 seconds. Wash in water. The above process is repeated three times. Following this the osmic acid solution is applied for 30 seconds, the specimen is washed in water, dried and mounted in balsam. The spiro-nemata bacteria and cellular detritus are stained black. This method is quick, reliable, and is to be recommended.

¹ Tunncliffe (Jour. Am. Med. Assn., 1912, LVIII, 1682) calls attention to a very simple and reliable method formerly used by Oppenheimer and Sachs as well as by Ploeger. Make smears as usual, fix in the flame and cover for a few seconds with a 10 per cent. mixture of a saturated alcoholic gentian-violet solution in 5 per cent. phenol. Wash in water and dry.

² Tilden (reported by Noguchi, Jour. Am. Med. Assoc., 1921, LXXVII, 2052) has originated a method of staining this organism with the Giemsa stain, as well as with basic dyes, such as gentian violet and fuchsin, which is simple and apparently reliable. A buffer solution containing formaldehyd (1 part of formaldehyd solution and 9 parts of phosphate buffer solution composed of 88 parts of $M/15$ Na_2HPO_4 12 parts of $M/15$ KH_2PO_4), is used as fixative, the scraping or tissue emulsion being suspended in a small amount of the buffered mixture and the mixture allowed to stand at least 5 minutes (the longer the fixation the better the results). Thin films are then made on clean slides, dried in the air, the film surface flooded with a saturated alcoholic solution of gentian violet or fuchsin and the slide is almost immediately washed in running water and air dried. If the Giemsa solution is employed as staining reagent, the slides are left in the fluid for 1 hour.

³ Jour. Am. Med. Assn., 1910, LIV, 1498.

India Ink Method.

Burri¹ has advanced this method, which is simple and reliable, although some specimens of ink may show confusing artefacts according to Barach,² who used an inferior ink. A drop of the fresh serum from the lesion is placed at one end of a glass slide and immediately mixed with a small drop of Gunther-Wagner India ink (Chin-Chin liquid pearl ink). The mixture is then spread and allowed to dry. The specimen is studied with the immersion lens. The whole field is a homogeneous brown or black color, the spironema, blood cells, etc., appearing as colorless highly refractile bodies. The morphology, length and numbers of spirals should distinguish the pallidum from the other types.³

Tribondeau's Method.

This⁴ is a modified and improved Fontana method and yields excellent results. It may be recommended for all routine staining of smears for the spironema, as it gives consistent and reliable microscopic pictures.

Solutions Necessary.

1. A fixing solution, consisting of 1 c.c. glacial acetic acid, 2 c.c. formalin, and distilled water 100 c.c.
2. A mordant consisting of 1 gram of tannin dissolved in 20 c.c. of distilled water. A small piece of camphor may be added to the solution to prevent contamination with molds.
3. The staining solution (Fontana's solution): Silver nitrate, 1 gram dissolved in 20 c.c. of distilled water. After solution is complete ammonia is added, drop by drop, until the precipitate first formed begins to dissolve and a faint opalescence remains.

Technic.

Films are made in the usual way, and dried in the air. Fix these films in the formol-acetic acid solution, which is poured on and off once or twice, a fresh quantity being left to act for 1 to 5 minutes. This procedure dissolves out the hemoglobin, which may be present in the blood corpuscles in the film. In the case of secretion of syphilitic ulcers, it is wise to wash the films with absolute alcohol before applying the fixative. Smears from organs should have the fat removed by alcohol, ether, and finally alcohol. After treatment with the fixing solution, cover the preparation with the tannin mordant and heat gently until vapor rises (do not boil). Allow the mordant to act for 30 seconds. Wash thoroughly with tap water for 30 seconds and follow with distilled water. Now pour on the silver solution and allow it to act for a few seconds in the cold. Pour this off and add a fresh quantity of the stain and heat gently until vapor arises. Allow the

¹ Das Tuschverfahren, Jena, 1909. See, also, Walters, Jour. Am. Med. Assn., 1914, LXIII, 1666.

² Jour. Am. Med. Assn., 1910, LV, 1892.

³ Goosmann (Jour. Cutan. Dis., 1911, XXIX, 628) has advised the use of nigrosin as a substitute for India Ink.

⁴ Bull. Soc. franc. de dermat. et Syph., 1912, XXIII, 474; Ann. de L'Inst. Pasteur, 1917, XXXI, 425. See Fontana, Dermat. Wchnschr., 1912, LV, 1003.

PLATE XXXIV.



FIGURE 11.

SPIROCHETÆ PALLIDÆ IN TISSUE. (LEVADITI'S STAIN.)

stain to act for a further 15 seconds, when the film is of a maroon color. Wash in distilled water for a few seconds and dry with blotting paper. The spironemata are stained yellowish brown or blackish. The stained films may be examined directly in cedar oil, but this should not be left on as the oil destroys the preparation. The film may be mounted, if desired, in Canada balsam.

Levaditi and Manouélian's Method.

This method,¹ also known as the pyridin method, is applied only to the detection of spironemata in tissues. It is superior to the older method of Levaditi. Cut the tissue into pieces about 1 to 2 mm. square, fix in 10 per cent. formalin for 24 hours and harden in absolute alcohol for 24 hours. Wash in distilled water until the pieces fall to the bottom of the vessel. Place the tissue in a ground-glass stoppered bottle and add 50 c.c. of the following mixture.

1 per cent. aqueous solution of silver nitrate,	90 c.c.
Pyridin,	10 c.c.

Leave them in the silver solution for two to three hours at room temperature and for three to five hours at 50°C. Wash in 10 per cent. pyridin solution. Immerse for three to four hours at room temperature in a freshly prepared solution with following formula:

4 per cent. aqueous solution of pyrogallol,	90 c.c.
Pure acetone,	10 c.c.
Pyridin,	15 c.c.

Remove from the reducing bath, dehydrate in absolute alcohol, clear in xylol and embed in paraffin. Cut sections and stain on the slide with a 2 per cent. aqueous solution of toluidine blue. Differentiate in absolute alcohol or Unna's ether-glycerin mixture, dehydrate in absolute alcohol, oil of bergamot and xylol. Mount in balsam and examine. The spironemata are stained beautiful black, while the ground-work of the tissue is blue.

Noguchi's Method.

This method² is used with beautiful results, especially with brain or cord tissue. Harden the tissue in 10 per cent. formalin for several days. The longer this is continued the better the results. Place the pieces (5 to 7 mm. thick) in the following mixture for five days at room temperature.

Formalin,	10 c.c.
Pyridin,	10 c.c.
Acetone,	25 c.c.
Alcohol (absolute),	25 c.c.
Distilled water,	30 c.c.

Wash thoroughly in distilled water for 24 hours. Transfer to 96 per cent. alcohol for three days and, then, wash in distilled water for 24 hours. Place

¹ Compt. rend. Soc. de biol., 1906, LX, 134. See, also, Sobernheim, Kolle and Wassermann's Handbuch d. pathog. Mikroorg., 1913, VII, 770.

² Jour. Cutan. Dis., 1913, XXXI, 547.

the tissue in an amber bottle and treat with 1.5 per cent. aqueous solution of silver nitrate for three days at 37°C. or five days at room temperature. Wash in distilled water for several hours. Reduce in the following mixture for 24 hours.

4 per cent. aqueous solution of pyrogallol,	95 c.c.
Formalin,	5 c.c.

Wash in distilled water, transfer to 80 per cent. alcohol for 24 hours, then to 95 per cent. alcohol for three days and absolute alcohol for two days. Clear in xylol, xylol-paraffin and embed in paraffin. Cut sections from various strata of the tissue. Sections 3 to 5 μ give the best results. The spirone-mata are pure black in color, while the tissue varies from a pale yellow to a yellowish-brown. The neuroglia fibers sometimes stain distinctly, but are brownish, never black.

Warthin and Starry's Method.

This method¹ is the second improved one of these authors and is more simple, more easily carried out and more accurate than their former method. It gives excellent results and may be relied on. The technic is as follows:

1. Tissues fixed in neutral formaldehyd. Thin slices of tissue, from 5 to 7 mm. thick, left in 4 per cent. neutral formaldehyd (10 per cent. formaldehyd solution) from one to three days give the best results. If tissues are put in alcohol before the formaldehyd fixation is complete, the results are less satisfactory. It is wise to fix these tissues as soon as removed from the body if possible.

2. Embed in paraffin.

3. Cut and mount sections on cover-glasses with albumin fixative. A minimum amount of this fixative should be spread evenly on the clean cover-glass, so that there will not be enough of it to become heavily stained. The prepared cover-glasses should be placed on a clean card-board, covered with clean paper and dried in the incubator for 12 hours before using.

4. Remove paraffin. After the mounted paraffin sections have been dried on the cover-glasses, the paraffin is removed by heating over the flame and dropping into xylol, followed by absolute alcohol and then distilled water.

5. The mounted cover-glass is taken from the distilled water rinsed in 2 per cent. silver nitrate solution, and then covered with another clean cover-glass also wet with the silver solution. The silver solution should always be freshly made and never used after it is from 3 to 4 days old. The two attached cover-glasses are then placed carefully in a brown wide-mouthed bottle so that they are on edge against the side of the bottle, and silver nitrate solution (2 per cent.) poured into the bottle so as not to cover them completely. Stopper the bottle tightly and place in the incubator for from 30 minutes to 1 hour. After impregnation, the cover-glasses are removed

¹ Jour. Am. Med. Assoc., 1921, LXXVI, 234. See, also, Am. Jour. Syph., 1920, IV, 97. For other methods of demonstrating spironemata in tissue, see Nardelli, Policlinico, 1919, XXVI, 1288; Schultze, Jour. Am. Med. Assoc., 1920, LXXV, 176; Haythorn, Ibid., 1921, LXXVI, 725.

from the bottle, separated and the mounted section put into the following reducing mixture, section side up, for a few seconds until reduction is noted

6. The composition of the reducing mixture is as follows:

	c.c.
2 per cent. silver nitrate solution.....	3
Warm glycerin.....	5
Warm 10 per cent. aqueous gelatin solution.....	5
Warm 1.5 per cent. agar suspension.....	5
5 per cent. aqueous hydroquinone solution.....	2

The warm glycerin and gelatin solutions and the silver nitrate solution are first thoroughly mixed, then the agar suspension is stirred in with a glass rod, and finally the hydroquinone added, just before the sections are placed in the mixture. The agar suspension must be carefully made; should it become flaky and settle out, it must be brought to the boiling point again with constant stirring. It should be just thin enough to pour and not too thin and watery. The amount of hydroquinone added varies somewhat with the tissue and the fixation. In general it is best to use as small an amount as possible so that the reduction is not too rapid. Usually from 0.5 to 1 c.c. gives the best results, but in some cases more may be required. After the hydroquinone has been added, the mixture is stirred vigorously for a short time and poured into a staining dish. The longer the tissue remains in the reducing mixture the darker it will be. When it becomes a light reddish brown it is removed and

7. Washed in a 5 per cent. aqueous solution of sodium thiosulphate, then rinsed in distilled water, dehydrated in absolute alcohol, cleared in pure xylol, and mounted in balsam. When the sections have been properly stained, they will be light reddish brown. If a deep brown or black, the tissue will be too deeply stained and the spirionemata not sufficiently contrasted. The spirionemata are dark reddish brown to jet black against a very light brown.

IV. CYTOLOGY

The cytology¹ of transudates and exudates has reference to the study of the various types of cells found in such fluids. As a rule, such investigations, known as cytodiagnosis, are carried out more frequently on the non-purulent types of fluid, as the examinations of the purulent fluids are more especially concerned with the bacteria present.

Technic.

The technic of obtaining the cellular components of the puncture fluid will vary according as the effusion does or does not contain fibrin. If this be present in fairly large amount, the fluid must first be defibrinated before the next steps are possible. Here, again, the procedure may be complicated by the presence or absence of coagula. If the fluid be not coagulated, it is placed in a large sterile flask which contains a few sterile glass beads, the mixture is actively shaken until a firm clot is obtained, in the meshes of which a few

¹ See Labbé, Cytodiagnostic, Paris, 1903; Ravaut, Cytodiagnostic, Paris, 1901; Brion, Centralbl. f. allg. Path., 1903, XIV, 609.

of the cells will necessarily be enclosed, but the majority will remain suspended in the liquid. The fluid is then separated from the clot and placed in centrifugal tubes which are drawn out to a rather fine point. If the fluid be coagulated when collected, it should be shaken with glass beads to break up the clot and liberate the cellular elements. The fluid is then separated and treated as follows.

The remaining portion of the technic is the same for fluids which contain or do not contain fibrinous material. The centrifuge is rotated rather rapidly for about ten minutes in order to collect the cells as a sediment. In most of the exudates the number of these cells is very small so that a concentration is absolutely essential. As Widal and Ravaut have shown, the polynuclear cells seem to be somewhat more affected by the defibrination than the other types, so that these cells may show a relative diminution. After the cells have collected, the fluid is removed by rapidly inverting the tube in such a way that the sediment does not follow the liquid. Some workers advise the withdrawal of the sediment by a pipet with a long fine tip, but the writer has not found this method any more advantageous than the one spoken of above. The sediment in the tube is shaken so as to mix it thoroughly and a drop placed upon the glass slide and spread as described under Blood. Where very few cells are present it is wise to allow the drop to dry on the slide without spreading, in order to concentrate the cells in a small area. The specimen is then treated with Wright's stain and examined under the immersion lens. If stains are to be used which contain no fixative, such as methyl alcohol, it is necessary that the specimen be fixed by methods previously discussed in the section on Blood. The stain to be used will depend largely upon the points to be studied, the eosin-hematoxylin stain being especially serviceable in differentiating the nuclear structures of the cell. As the cells in the various pathologic fluids frequently show more or less degeneration, the nuclear portion is more suitable for study than is the cell protoplasm. For this reason the writer prefers the use of the eosin-hematoxylin method along with the Wright stain or the triacid stain for the granules of the cells.

These specimens may also be used for the study of the bacteria present. When this object is to be subserved, it is advisable to make two specimens, staining one with the ordinary methylene blue stain and a second by Gram's stain. If the material be very limited in amount, it is possible to combine the above staining methods by treating first with the eosin-hematoxylin method, washing in water and then following the ordinary procedure of the Gram method of staining. Such specimens are extremely panoptic and are especially to be recommended.

Cytology of Normal Fluids.

The number of cellular elements in fluids from the various serous cavities of the body may vary from a very few to a large number. The cells observed are the red and white corpuscles of the blood, the latter of which are usually relatively more numerous than in the circulating blood and are usually largely of the polynuclear type, although mononuclear forms are frequently present.

Neutrophiles and eosinophiles are present under normal conditions, the latter being relatively more abundant than in the blood. If a large number of red cells are found, an injury of the small vessels during puncture usually explains their presence. Besides these types of cell, which are exactly similar to those of the blood, a few endothelial cells are practically always seen. These cells are observed of different shapes, may be single or grouped in sheets, and may be very much degenerated. They are larger than the other cellular elements, their contour is usually circular, but may be irregular; they are mononuclear, and frequently contain round vacuoles.

In examining normal as well as pathologic fluids for their cellular content, 100 cells should be counted if possible and the percentage of each type thus determined. This constitutes the cytologic formula of the exudate.

Cytology of Pathologic Fluids.

According to the theory of Metchnikoff, the presence of a bacterial infection is associated with attraction of the leucocytes to the infected area. These cells then enter into combat with the bacteria and either destroy the organism or are destroyed by them. As has been shown, certain organisms, especially the tubercle bacillus and probably the typhoid organism, attract the lymphocytes, while most of the other organisms attract the polymorphonuclear neutrophiles. Theoretically, therefore, it should be possible to decide as to a tubercular or nontubercular condition by the presence or absence of an increased number of the mononuclear types of leucocytes. This is the basis of the attempt at differential diagnosis by means of cytodagnosis.

Not infrequently one finds in malignant conditions the so-called specific cells which are either sarcomatous or carcinomatous. These, although specific, are not easy absolutely to identify. These cells are very large, frequently showing fatty degeneration, extensively vacuolated, and showing a mitotic mulberry-like nucleus. Although differing from the endothelial cell, confusion is very apt to arise, so that it is difficult to make a diagnosis in all cases from the appearance of such cells.

Pleural Exudates.

Primary Tubercular Pleurisy.

This is characterized especially by an increase, both relative and absolute, in the number of lymphocytes. A pleural lymphocytosis exists when there is an excess of mononuclear cells, with abundant protoplasm, a large nucleus, and smaller than the endothelial cells. In the very early days of the infection a neutrophilia may exist, but this is rarely seen, as attention may not be drawn to the condition sufficiently early. Associated with these polynuclear cells in the early stage we may find an increase in the number of endothelial cells. Eosinophile cells are frequently observed, but do not have any definite relation to tuberculosis as an infection.¹ The red cells may be occasionally numerous, but are usually small in number.²

¹ Morris, *Jour. Lab. and Clin. Med.*, 1916, I, 540; Page, *Lancet*, 1920, I, 585.

² See Bullock and Twichell (*Am. Jour. Med. Sc.*, 1915, CXLIX, 848) for a study of exudates in artificial pneumothorax.

Secondary Tubercular Pleurisy.

As a rule, tubercular pleurisy secondary to a pulmonary tuberculosis yields a liquid which is poor in cells, practically all of which are very much altered and in some cases very difficultly recognizable. The polynuclear types may predominate to such an extent that a distinct polynucleosis exists as evidence of a septic rather than a true tubercular pleurisy. The polynuclear cells are usually old, much deformed, and their nature recognizable only by staining their neutrophile granules. Where the infection is directly tubercular and not mixed (the latter, however, usually being the case), an approximately equal division of the polynuclear and mononuclear forms may obtain. In this type of pleurisy the eosinophile cells may be very numerous, in one case of Widal and Ravaut constituting 54 per cent. of the cellular elements. The endothelial cells may be, as in the primary tubercular pleurisy, sufficiently numerous to constitute a distinct endotheliosis. They are, however, single and very rarely grouped in masses.

Pneumococcus Pleurisy.

This is a truly septic type of pleurisy and is characterized by a distinct polynucleosis.¹ In the early stages of this type of pleurisy, the endothelial cells may be very numerous, while in the later stages they may be much diminished. In this, as in all types of pus accumulations, marked autolysis is present so that the cells may show extreme degeneration. As this condition progresses toward recovery, some of the polynuclears may be replaced by the mononuclear lymphocytes; while if suppuration becomes extensive the polynuclears increase and autolysis becomes extremely marked. In such exudates the pneumococcus may be demonstrated by staining methods.

Streptococcus Pleurisy.

This type is especially associated with a polynucleosis. These cells are frequently observed undergoing karyolysis, the cell body usually being markedly degenerated. In its early stage it may be accompanied by an endotheliosis, but when well developed is usually associated with the presence of only a few isolated endothelial cells. Stained smears show streptococci in large numbers.

Typhoid Pleurisy.

In this type a lymphocytosis is usually observed along with an endotheliosis, these latter cells being in large masses instead of in single isolated forms as observed in the secondary tubercular pleurisy.² This point may be valuable in differentiating these two conditions, which are associated with an increase in the number of lymphocytes. The eosinophiles may be increased and red cells may be present in large numbers. The identification of the specific organism will serve as a positive differentiation from tubercular conditions.

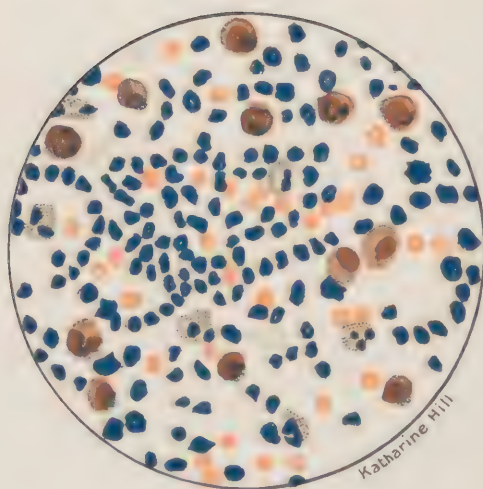
Malignant Pleurisy.

This is a type of the aseptic pleurisies and may accompany malignant growths of the lung or pleura. Nothing characteristic is found in the cytology.

¹ Evans, Jour. Infect. Dis., 1916, XIX, 440; Weiss, Arch. Int. Med., 1919, XXIII, 395.

² See Pepper, Am. Jour. Med. Sc., 1916, CLI, 663.

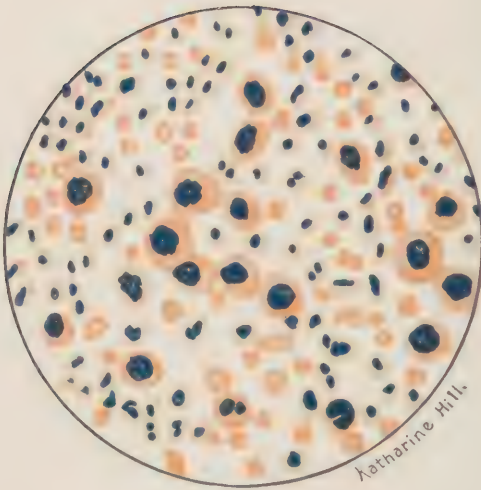
PLATE XXXV.



Katherine Hill

EXUDATE FROM TUBERCULAR PLEURISY. (EOSIN-HEMATOXYLIN STAIN)

PLATE XXXVI.



Katherine Hill.

EXUDATE IN PNEUMONIC PLEURISY. (EOSIN-HEMATOXYLIN STAIN.)

gic formula of such exudates, but occasionally portions of the tumor mass may be obtained or certain cells may be found which are more or less distinctive, although not absolutely pathognomonic. These cells are frequently confused with the larger endothelial cells of the pleura, but are characterized, according to Deguy and Guillaumin, as follows: Malignant pleural cells contain glycogen which is recognized by the brown coloration shown on treatment with dilute iodine solutions, they contain large amounts of fatty material, they are extensively vacuolated, these vacuoles may be large or so numerous and small that the cell resembles a sponge, the cells are extremely large, and the nucleus usually shows mitotic figures.¹

Nephritic and Cardiac Pleurisy.

The secondary exudate observed in cardiac and renal conditions is characterized especially by the marked endotheliosis. These endothelial cells are grouped in masses of 5 to 10 cells, which show more or less degeneration, their contours and their large nuclei being distinct. This endotheliosis is not complicated by the presence of many other cells in the pure nephritic pleurisy, but in the cardiac type we usually find a polynucleosis at the same time. This polynucleosis is much more marked where infarcts or emboli have complicated the condition than when it is due to a pure congestion. Numerous red cells are especially observed in association with a congestive pleurisy.

Peritoneal Exudates.

The cytological examination of peritoneal exudates has, as yet, yielded fewer diagnostic points than has that of pleural exudates.² It is, however, sometimes possible to differentiate a tubercular peritonitis from an ascites or an ovarian cyst by means of such examinations. Tubercular peritonitis usually shows a lymphocytosis and also a relative polynucleosis. A few endothelial cells may occasionally be found, but these do not yield much information. In ascites of hepatic origin few cellular elements are observed beyond the peritoneal endothelial cells. In ovarian cysts there are fewer cellular elements as a rule, but those present are usually large, round, or oval, and filled with vacuoles. Moreover, cylindrical ciliated epithelial cells as well as goblet cells or red cells are frequently relatively numerous.

V. CYST FLUIDS

(1) Ovarian Cysts.

(a) Serous Cysts.

Such cysts are true retention cysts formed by dilatation of the Graafian follicles and retention of the ovarian secretion and are known as *Hydrops folliculorum Graafii*. They contain a clear, watery, serous liquid, which has an amber color, a specific gravity ranging between 1.005 and 1.022, and a chemical composition practically identical with that of other serous fluids.

¹ See Warren, Arch. Int. Med., 1911, VIII, 648; Josefson, Ztschr. f. klin. Med., 1916, LXXXII, 331; Judd, Am. Jour. Med. Sc., 1917, CLIII, 717.

² See Szeesi, Folia Hæmat., 1912, XIII, 1. See Rolleston (Brit. Med. Jour., 1914, I, 238) and Weishaupt (Arch. f. Gynäk., 1914, C, 496) for a discussion of eosinophilia in peritoneal exudates; also, Carslaw, Brit. Jour. Surg., 1915, III, 1.

(b) Myxoid or Colloid Cysts.

These are proliferating cysts developed from the epithelial tubules. "We sometimes find in small cysts a semisolid, transparent, or somewhat cloudy or opalescent mass which appears like solidified glue or quivering jelly, and which has been called colloid because of its physical properties. In other cases the cysts contain a thick, tough mass which can be drawn out into long threads, and, as this mass in the different cysts is more or less diluted with serous liquids, their contents may have a variable consistency. In other cases the small cysts may also contain a thin watery fluid. The color of the contents is also variable. In certain cases it is bluish-white, opalescent, and in others yellow, yellowish-brown, or yellowish with a shade of green. They are often colored more or less chocolate-brown or reddish-brown, due to the decomposed blood pigment. The reaction is alkaline or nearly neutral. The specific gravity, which may vary considerably, is generally 1,015 to 1,030, but may be, in a few cases, 1,005 to 1,010 or 1,050 to 1,055. Though the contents of the proliferating cyst may have a variable composition, still it may be characterized, in typical cases, by its slimy or ropy consistency; by its grayish-yellow, chocolate-brown, sometimes whitish-gray color; and by its relatively high specific gravity. Such a liquid does not ordinarily show a spontaneous coagulation" (Hammarsten).

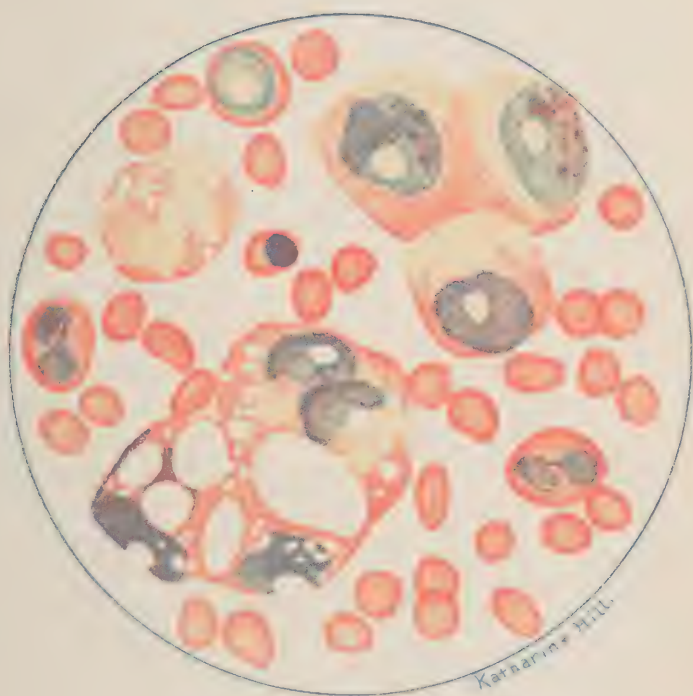
Microscopical examination of the sediment shows red and white blood-cells, large epithelial cells, which may be filled with vacuoles, cylindrical or goblet cells, granular cells showing more or less fatty degeneration, fatty granules, cholesterin crystals, and colloid corpuscles in the form of large, circular, highly refractile bodies.¹

Chemically, these cysts are characterized by the presence of colloid, which is not a distinct chemical entity. It is a gelatinous substance, insoluble in water and acetic acid, soluble in alkalis, and yields a reducing body on boiling with acids. Not infrequently pseudomucin (metalbumin) is found, especially in the extremely viscid fluids. For its detection the serum albumin must be previously removed by the addition of acetic acid, boiling, and filtering. The filtrate is treated with alcohol when a thready precipitate forms. If this precipitate be boiled with HCl, a substance is formed which reduces copper solutions quite markedly. Pseudomucin is distinguished from true mucin by the fact that it is not precipitated by acetic acid. Mitjukoff has isolated a further colloid body from certain ovarian cysts, to which he gives the name of paramucin. It is precipitated by acetic acid and is soluble in an excess. If treated with alkali it first swells up and then dissolves in an excess of the reagent. It differs from mucin and pseudomucin in the fact that it reduces copper solution without previous boiling with acids. McConnell² has recently reported the finding of a multi-locular cyst of the ovary, which contained true mucin.

¹ Dienst (Münch. med. Wchnschr., 1912, LIX, 2731) has called attention to the fact that fibrin is absent in the fluid from ovarian cysts while it is constantly present in ascitic fluids. Arnold (Jour. pharm. chim., 1920, XXI, 305) reports a case in which cholesterol was the chief constituent of the cyst. Harley (Indian Med. Gaz., 1921, V, 18) reports the case of an ovarian cyst weighing 164 pounds.

² Jour. Med. Research, 1909, XX, 105.

PLATE XXXVII.



EXUDATE IN MALIGNANT PLEURISY. (EOSIN-HEMATONYLIN STAIN. AFTER DEGUY AND GUILLAUMIN.)

(c) **Papillary Cysts.**

These are intraligamentary types and contain a yellow, yellowish-green, or brownish-green fluid which contains only traces of pseudomucin. The specific gravity ranges between 1,032 and 1,036.

(d) **Dermoid Cysts.**

These may be seen in the shape of small cysts not larger than a pea, but usually they are much larger, in some cases reaching the size of a man's head. The cyst usually contains a fatty unctuous material, which is derived from the epidermal lining of the cyst, and associated with it fat, desquamated epithelial scales, hair, teeth, bone, cartilage, etc.¹

(2) **Parovarian Cysts.**

These cysts of the organ of Rosenmüller contain a clear, pale yellow, or colorless, limpid fluid or occasionally one showing slight opalescence. The specific gravity ranges from 1,002 to 1,010 and differs from that of the ovarian cyst by its usual limitation to these lower figures. Albumin may be present in small amounts or be entirely absent, while pseudomucin is rarely, if ever, present.

(3) **Hydrocele.**

The contents of such a cyst are usually clear, show a color which may range from yellow to green, have a specific gravity of 1,015 to 1,030, and usually coagulate spontaneously. They contain a relatively large percentage of albumin, of which about 50 per cent. is globulin. In ordinary hydrocele many large oval cells may be seen, which have an eccentric nucleus and may be grouped in masses, although more frequently they appear as isolated cells; in some cases many eosinophiles, but this is rare in uncomplicated hydroceles. If the hydrocele be of tubercular origin a marked lymphocytosis usually exists.

(4) **Spermatocele.**

Fluids from such cysts are usually thin, colorless, and cloudy like thin milk. They may have an acid reaction, but are ordinarily alkaline. The specific gravity ranges between 1,006 and 1,010. Such fluids do not coagulate either spontaneously or after the addition of blood. Microscopically, one observes cell detritus, fat granules, leucocytes, and spermatozoa.

(5) **Hydronephrosis.**

This is a true retention cyst of the kidney, due primarily to obstruction of the ureter, which may be either congenital or acquired. Material aspirated from a renal cyst is usually clear, but may be yellowish or reddish and distinctly turbid. Its specific gravity varies from 1,010 to 1,015, while the chemical composition is usually suggestive of urine. For some time the presence of urea and uric acid in hydronephrotic cysts was supposed to be pathognomonic, but it has been shown that these substances may be present both in ovarian

¹ See Spalding, *Am. Jour. Obs. and Dis. Women and Child.*, 1919, LXXX, 401.

and pancreatic cysts and may even be lacking in old renal cysts. Occasionally epithelial cells, derived from the uriniferous tubules may be found, but such cells are not always present nor are they sufficiently characteristic to be of great importance from the diagnostic standpoint.

(6) Hydatid Cysts.

The fluid obtained by puncture of an echinococcus cyst is usually clear and of an alkaline reaction, has a specific gravity varying between 1,005 and 1,015, is practically free from albumin, and contains a large amount of inorganic salts, especially of sodium chlorid. The characteristic findings of such a cyst are the hooklets, scolices, and shreds of faintly laminated membrane. In some cases no trace of any morphological elements can be found, but usually the diagnosis is rendered certain, especially if careful search be made, by the presence of some portion of the parasite or the cyst membrane.

(7) Pancreatic Cysts.

The puncture fluid from a pancreatic cyst varies in its physical properties, depending upon the nature of the cyst as well as the length of time the fluid has remained in the cyst. It is usually bloody in character, has a specific gravity ranging from 1,010 to 1,030 and may contain methemoglobin, hematin, and cholesterin. As characteristic constituents of such a cyst one finds ferments, which will digest all types of food material.¹ Such tests may be performed as outlined in the sections on Gastric Contents and Feces.

VI. CEREBROSPINAL FLUID

Since the introduction of lumbar puncture by Quincke, the cerebrospinal fluid has gained more or less importance from the diagnostic point of view. So much may be learned, either from the standpoint of direct or differential diagnosis, that every practitioner should be able to perform a lumbar puncture and to examine the fluid obtained. There is little danger in the procedure as the spinal cord does not reach to the point of puncture and the fibers of the cauda equina are sufficiently movable to escape the needle. While few bad effects are observed in the ordinary run of cases, a few have been reported in which symptoms of collapse were evident. It should be a rule, therefore, to stop proceedings if such symptoms arise and also to keep the patient quiet in bed for at least 24 hours following the puncture, so that the pressure in the cerebrospinal cavity may become equalized.²

¹ See Christian (*Arch. Int. Med.*, 1912, IX, 143) who reports peculiar findings in the fluid of an epigastric cyst; also Besley, *Jour. Am. Med. Assn.*, 1914, LXII, 1011; Ipsen, *Hospitaltid.*, 1914, LVII, 889 and 921; Speese, *Ann. Surg.*, 1914, LX, 673; Rosenthal, *Arch. f. Verdauungskr.*, 1914, XX, 619; Eustachio *Rif. Med.*, 1915, XXXI, 424; Willis and Budd, *Surg., Gyn. and Obs.*, 1915, XX, 688; Gilbride, *Jour. Am. Med. Assoc.*, 1920, LXXV, 149.

² For discussions regarding the source of the spinal fluid and variations in it see Dixon and Halliburton, *Jour. Physiol.*, 1913, XLVII, 215; *Ibid.*, 1914, XLVIII, 129 and 317;

Lumbar Puncture.

The patient is placed upon his left side near the edge of the bed, the knees should be flexed upon the abdomen, and the site of puncture prepared as for any surgical procedure. The needle used for puncture should be from 5 to 10 cm. long and have a lumen from 1 to 2 mm. in diameter. It is always wise to provide the larger needles with a stylet, so that tissue fragments or blood may not gain entrance accidentally to the tube and thus lead to possible diagnostic errors. This stylet may be removed the moment the needle penetrates the dura mater.

The site of puncture should be on a level with the junction of the third and fourth lumbar vertebræ at a point about 1 cm. to the side (preferably the upper) of the median line. The needle is directed slightly upward and inward, the depth to which the puncture should be made varying with the age of the patient, the younger the child the less the depth. This puncture should be made carefully and yet with sufficient force to penetrate easily the musculature. If any marked resistance arises, it is probable that the needle has struck the vertebra, in which case the pressure must be reduced or the needle may break. This is not an infrequent occurrence with those not used to the technic, so that it may be advisable for the student to practice the procedure upon the cadaver. As soon as the dural sac is reached, the cerebrospinal fluid will flow from the canula, the rate of flow indicating in a general way the pressure of the fluid. No aspiration should be used at any time, as this procedure is extremely dangerous.

It is frequently advisable to know exactly what this pressure is, so that one may resort to the following method as used by Sahli. As soon as the needle penetrates the dura, a connection is made with a mercury manometer by means of a rubber tube filled with a 1 per cent. solution of carbolic acid. The portion of the manometer above the level of the mercury, forming the connection between it and the carbolic acid tube, must also be filled with the fluid. The manometer is filled with mercury to the zero point and held in such a manner that this point is on a level with the point of the aspirating needle, which is possible with ordinary manometers only when the connecting tube is of considerable length.¹ Under normal conditions the dural pressure, in the dorsal position, ranges between 5 and 7.5 mm. of mercury, or 60 to 100 mm. of water if a water manometer be used. In pathologic conditions, such as meningitis or brain tumor, it ranges between 15 and 60 mm. of mercury or 200 to 800 mm. of water.

Dandy and Blackfan, *Jour. Am. Med. Assn.*, 1913, LXI, 2216; Frazier, *Ibid.*, 1914, LXIII, 287; Cushing, *Jour. Med. Research*, 1914, XXXI, 1; Weed, *Ibid.*, 21, 51 and 93; Wegefarth, *Ibid.*, 119 and 149; Wegefarth and Weed, *Ibid.*, 167; Frazier and Peet, *Am. Jour. Physiol.*, 1914, XXXV, 268; Weed and Cushing, *Ibid.*, 1915, XXXVI, 77; Frazier, *Jour. Am. Med. Assn.*, 1915, LXIV, 1119; Halliburton, *Brain*, 1916, XXXIX, 213; *Lancet*, 1916, II, 779; Bloch, *Jour. A. M. A.*, 1917, LXVIII, 691; Becht, *Am. Jour. Physiol.*, 1920, LI, 1 and 126; Stepleanu-Horbatsky, *Presse Méd.*, 1920, XXVIII, 254; Barré and Schrapf, *Bull. Méd.*, 1921, XXXV, 63; Stern and Gautier, *Arch. intern. physiol.*, 1921, XVII, 138; Stern, *Schweiz. Arch. f. Neurol. u. Psychiat.*, 1921, VIII, 215; Monakow, *Ibid.*, 233.

¹ See Strauss (*Jour. Am. Med. Assn.*, 1914, LXII, 1327) for a special spinal-puncture needle with manometer attachment.

Physical Properties.

Normal cerebrospinal fluid is colorless, limpid, and free from morphological elements. Its specific gravity ranges between 1,002 and 1,010.¹ It is alkaline in reaction, the degree of alkalinity varying between 15 and 20.² It contains a trace of protein and about 0.1 per cent. of glucose. The salt content of

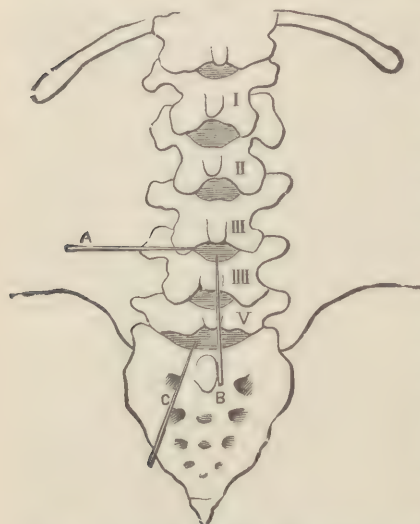


FIG. 160.—Lumbar puncture: A, Quincke's site; B, Maran's site; C, Chipault's site. (Tyson.)

this fluid is, according to Zdarek, 0.836 gram, of which 0.429 gram is referable to sodium oxid and 0.017 to potassium oxid. This would rather militate against the older statements that a large amount of potassium salts as compared with sodium salts was present. The relation of KCl to NaCl is, according to Nawratzki, 1 to 18, while Zdarek gives this ratio as 1 to 40. The former figure agrees closely with that obtained by various workers with pathological cerebrospinal fluids.³ The amount of fluid obtained by lumbar puncture is extremely variable. In normal individuals this amount is unknown as puncture is rarely performed upon normal cases. Pathologically, the amount obtained varies between 10 and 100 c.c. Naturally, if

the communication between the subarachnoid spaces of the brain and of the spinal cord is blocked by a tumor or inflammatory adhesions, or if the aqueduct of Sylvius or the foramen of Magendie be obliterated, little fluid may be obtained by puncture, although large amounts may be present above the obstruction. The largest amounts are seen in cases of serous or tubercular meningitis, so that such conditions may usually be ruled out if a small amount of fluid is obtainable.

Pathologically, we may observe a very cloudy fluid, due to the presence of leucocytes, erythrocytes, and endothelial cells. This cellular admixture may be so extensive that the fluid resembles pure pus. In cases of cerebral hemorrhage from the ventricles, hemorrhagic pachymeningitis, or traumatic lesions of the spinal cord, so much blood may be present as to give the appearance of practically pure blood, the color varying from a bright red to a brownish or greenish-red, depending upon the length of time it has remained in con-

¹ See Stanford (Ztschr. f. physiol. Chem., 1913, LXXXVI, 43 and 219), who shows that this density is higher in progressive paralysis and epilepsy than in other conditions.

² Levinson (Jour. Infect. Dis., 1917, XXI, 556) shows that the H-ion concentration (PH) of the spinal fluid in nonmeningitic cases varies between 7.4 and 7.6. See, also, Levinson, Arch. Pediat., 1916, XXXIII, 241; Hurwitz and Troutner, Arch. Int. Med., 1916, XVII, 828; Felton, Hussey, and Bayne-Jones, Ibid., 1917, XIX, 1085; Meier, Biochem. Ztschr., 1921, CXXIV, 137; Shearer and Parsons, Quart. Jour. Med., 1921, XIV, 120.

³ See Rosenbloom and Andrews, Arch. Int. Med., 1914, XIV, 536; Halverson and Bergheim (Jour. Biol. Chem., 1917, XXIX, 337) discuss the calcium content of the cerebrospinal fluid. See, also, Rodillon, Bull. Sci. Pharmacol., 1920, XXVII, 249.

tact with the remaining portion of the fluid. This admixture with blood may lead to the spontaneous coagulation of the fluid.¹ This may serve as a differentiating point between inflammatory and noninflammatory lesions. Thus, in tubercular meningitis very slight coagulation may be observed while in the epidemic cerebrospinal meningitis the coagulum may be very firm.

Chemical Properties.

The chemical examination of the cerebrospinal fluid offers some points of clinical value.² While the albumin content normally is much less than 0.1 per cent., it may vary under pathologic conditions to as high as 0.8 per cent. The total protein, especially the euglobulin portion, is increased in all cases of acute exudative inflammations of the meninges, in hydrocephalus as Polanyi³ has shown and, also, in syphilitic and parasymphilitic diseases of the cerebrospinal tract. Glucose is usually present but may entirely disappear under pathologic influences due to the autolysis controlled by the leucocytic ferments, the glucose being converted into lactic acid.⁴ Cholin is present nor-

¹ Schwarz (Deutsch. Ztschr. f. Chir., 1913, CXXIV, 346) shows that a yellowish tint (xanthochromasia) is frequently due to conversion of the blood pigment into urobilin and, hence, that this is a valuable indication of intracranial hemorrhage. See, also, Babes, *Compt. rend. Soc. de biol.*, 1914, LXXVII, 67; Hanes, *Am. Jour. Med. Sc.*, 1916, CLII, 66; Sprunt and Walker, *Bull. Johns Hopk. Hosp.*, 1917, XXVIII, 80; Lantuéjoul, *Rev. Neurol.*, 1920, XXVII, 339; Wallgren, *Acta Med. Scandin.*, 1920, LIII, 303; Levison, *Arch. Int. Med.*, 1920, XXVI, 459; Abt and Tumpeer, *Am. Jour. Dis. Child.*, 1920, XX, 153; Arias, *Arch. de Neurobiol.*, 1920, I, 416; Leschke, *Deutsch. med. Wchnschr.*, 1921, XLVII, 376. For total nitrogen and urea of spinal fluid see Adler and Ragle, *Boston, Med. and Surg. Jour.*, 1914, CLXXI, 769; Woods, *Arch. Int. Med.*, 1915, XVI, 577; and Cullen and Ellis, *Jour. Biol. Chem.*, 1915, XX, 511; Kahn, *Ibid.*, 1916, XXVIII, 203. See Morse and Crump (*Jour. Lab. and Clin. Med.*, 1919, V, 185) for a method for estimation of preformed ammonia in spinal fluid. Weston (*Jour. Med. Research*, 1915, XXXIII, 119), discusses the cholesterol variations in this fluid. See, also, Fabris *Pediatr.*, 1921, XXIX, 1057; Levinson, *Landenberger and Howell, Am. Jour. Med. Sc.*, 1921, CLXI, 561.

² See Cantieri, *Rif. Med.*, 1916, XXXII, 977 and 1009; Kahn and Neal, *Proc. Soc. Exper. Biol. and Med.*, 1916, XIV, 26; Johnston, *Am. Jour. Dis. Child.*, 1916, XII, 112; Leopold and Bernhard, *Ibid.*, 1917, XIII, 34; Levinson, *Am. Jour. Dis. Child.*, 1919, XVIII, 568.

³ *Biochem. Ztschr.*, 1911, XXXIV, 205. See, also, Ferrier, *Lancet*, 1913, II, 1107; Ayer and Viets, *Jour. A. M. A.*, 1916, LXVII, 1707; McGregor, *Jour. Biol. Chem.*, 1917, XXVIII, 403; Novick, *Jour. Infect. Dis.*, 1917, XXI, 52; Lécène, Mestrezat and Boutillier, *C. R. soc., biol. Paris*, 1918, LXXXI, 597.

⁴ See Kopetzky (*Trans. Am. Acad. of Ophth. and Oto-Laryngol.*, 1912, I, 261) who believes the absence of the copper-reducing body to be the earliest sign of bacterial invasion of the central nervous system. See Szabo, *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1913, XVII, 145; Soper and Granat, *Arch. Int. Med.*, 1914, XIII, 131; Hopkins, *Am. Jour. Med. Sc.*, 1915, CL, 847; Schloss and Schroeder, *Am. Jour. Dis. Child.*, 1916, XI, 1; Rieger and Solomon, *Boston Med. and Surg. Jour.*, 1916, CLXXV, 817; Kraus and Corneille, *Jour. Lab. and Clin. Med.*, 1916, I, 685; Kiely, *Ibid.*, 1917, II, 645; Weil, *C. R. Soc. biol. Paris*, 1918, LXXXI, 364; Mestrezat, Weissenbach and Boutillier, *Ibid.*, 655; Weissenbach, *Bull. et Mem. Soc. Méd. hôp. de Paris*, 1918, XXXIV, 113; De Laverne, *Ibid.*, 1920, XXXVI, 1246; Rouquier, *Ibid.*, 1375; Goto and Hayashi, *Jap. Med. World*, 1921, I, 23; Egerev-Seham and Nixon, *Arch. Int. Med.*, 1921, XXVIII, 561; Stevenson, *Arch. Neurol. and Psychiat.*, 1921, VI, 202; Briand and Rouquier, *Bull. de la Soc. Med. des Hop.*, 1921, XLV, 145; Kasahara and Hattori, *Am. Jour. Dis. Child.*, 1921, XXII, 218; Coope, *Quart. Jour. Med.*, 1921, XV, 1 (shows that a high sugar should not be regarded as a positive diagnostic sign of lethargic encephalitis and that a low sugar content is very significant of an acute or tuberculous meningitis); Kahler, *Wien. klin. Wchnschr.*, 1922, XXXV, 8. The methods to be followed in determination of the sugar are either the Folin and Wu or the Benedict technics as discussed under blood. Genoese (*Pediatrics*, 1920, XXVIII, 449) reports strong reactions for acetone in the spinal fluid in cases of tuberculous meningitis, while Koopman (*Nederl. Tijdschr. v. Geneesk.*, 1920, I, 1346; *Deutsch. med. Wchnschr.*, 1920, XLVI, 489) obtained it in diabetes and occasionally in epilepsy.

mally in traces, while pathologically it may vary, according to Donath, between 0.021 per cent. and 0.046 per cent.

Koch¹ has introduced the following method for examination of this fluid, which should extend our knowledge. The fluid is centrifuged and the sediment used for the bacteriological and cytological examinations. Ten c.c. of this clear fluid are placed in a beaker, 3 c.c. of a saturated solution of ammonium nitrate and 5 drops of nitric acid are added, and the mixture heated on a water-bath until complete coagulation has occurred. This precipitate of protein² is filtered through a perforated Gooch crucible, washed with 0.1 per cent. ammonium nitrate solution and then with alcohol, dried at 100°C. and weighed. The filtrate is taken before the washing with alcohol is begun, concentrated to 10 to 15 c.c. and 2 c.c. of 1 per cent. barium nitrate solution added while boiling. The barium sulphate precipitate is allowed to settle over night, is filtered off, dried and weighed. With the filtrate a phosphorus estimation is made by the molybdic method, with subsequent precipitation as magnesium ammonium phosphate in the usual way. Increase in the phosphorus and sulphur content is associated with degenerative changes in the nerve tissue. A test for reducing substances is made with 1 c.c. of the original fluid.

Microscopic Examination.

By far the most important part of the clinical examination of cerebro-spinal fluid is a study of the bacteriology and cytology of the fluid. Normally the fluid contains practically no morphologic elements, while under pathologic conditions large numbers of various types of cells may be present. The material for examination is obtained as previously described in the section on cytology. In true tubercular meningitis a lymphocytosis is almost invariably observed, while in the epidemic type, due to the meningococcus of Weichselbaum, the cells are of the polynuclear type. In the chronic cases of epidemic meningitis as well as during convalescence from this disease, the lymphocytes may be present to such an extent that a slight degree of lymphocytosis is present, but never to the same extent as observed in the tubercular type.³ A lymphocytosis is also observed in syphilitic lesions of the central nervous system. This is important from the standpoint of differential diagnosis. In certain cases of brain tumor, of non-specific origin, a lymphocytosis may obtain along with an increase in the protein-content of the fluid. In the meningitis due to the pneumococcus, a polynucleosis is the rule, although occasional cases are seen in which a lymphocytosis obtains.⁴ In the prodromal stages and early days of acute anterior poliomyelitis a marked

¹ Arch. Neurol., 1907, III, 331; Arch. Neurol. and Psychiat., 1909, IV, 1; Koch and Koch, Jour. Biol. Chem., 1917, XXXI, 395.

² Mott advises the following method for protein: 10 c.c. of the centrifuged fluid are faintly acidified with acetic acid and 20 c.c. of absolute alcohol are added. The mixture is boiled and filtered, the precipitate of protein being dried and weighed.

³ Wassermann and Lange (Berl. klin. Wchnschr., 1914, LI, 527) believe that these lymphocytes are, at least, one source of the substances responsible for the positive Wassermann reactions obtained with spinal fluid.

⁴ See, however, Brady, Jour. Am. Med. Assn., 1913, LX, 972. Barker (Southern Med. Jour., 1921, XIV, 437) reports apparently the first case in which cells of leukemic origin (myelocytes) were demonstrated in the spinal fluid. See, also, Levinson, Jour. Lab. and Clin. Med., 1922, VII, 626.

increase of fluid is accompanied by an increase in number of cells, which are largely of the mononuclear type. These are replaced by the polymorphonuclear forms as the number diminishes. In the microscopic examination of the cerebrospinal fluid it is sometimes of interest to know the number of cells per c. mm., of the fluid. The technic is as follows: The following mixture is employed for staining the white and dissolving the red cells: Methyl violet 0.2 gram, glacial acetic acid 5 c.c., water to 100. Fill the pipet with stain to the mark 1 and then fill to point 11 with uncentrifuged cerebrospinal fluid. Shake thoroughly, let stand for five minutes and count, using a Fuchs-Rosenthal counting chamber. The ruled surface of the cell contains 3.2 c. mm. of fluid. Count all of the white cells in the entire ruled area, multiply by 11 and divide by 32. The result is the number of cells per c. mm., which varies from 1 to 10 normally.¹

Epidemic Cerebrospinal Meningitis.

In these cases the fluid may be transparent, but is usually somewhat opalescent and may be thick and purulent.² The cellular elements are usually polynuclear in type and red cells may be more or less numerous.

Smears made from the sediment show the presence of numerous diplococci, which closely resemble the gonococcus in morphological and staining characteristics. This organism, the diplococcus meningitidis intracellularis of Weichselbaum, appears as a diplococcus, each element forming a hemisphere with its parallel side contiguous to that of its mate.³ It is sometimes seen in the form of tetrads or as isolated cocci, which appear as true spheres of variable size and showing a clear space in their interior. It is stained with the ordinary dyes and is negative to Gram's stain. For its cultural peculiarities see the last chapter of this book. Not infrequently one observes specimens of the meningococcus which show a Gram-positive reaction, so that it is difficult to distinguish them, especially when they are in the form of isolated cocci or in groups of two or four, from the pneumococcus. This type has been described as the meningococcus of

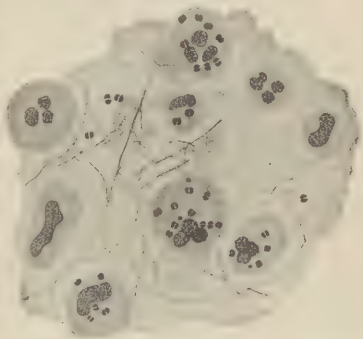


FIG. 161.—*Diplococcus intracellularis meningitidis*. (Councilman.)

¹ See Blatteis and Lederer, *Jour. Am. Med. Assn.*, 1913, LX, 811; Karpas, *Ibid.*, 1913, LXI, 262; Engman, Buhman, Gorham and Davis, *Ibid.*, 735; Brem, *Ibid.*, 742; Maruyama, *Wien. klin. Wchnschr.*, 1913, XXVI, 1233; Ball, *Interstate. Med. Jour.*, 1913, XX, 1109; Rubenstone, *New York Med. Jour.*, 1913, XCVIII, 1210; Gordon, *Ibid.*, 1914, XCIX, 110; Mitchell, Darling and Newcomb, *Jour. Nervous and Ment. Dis.*, 1914, XLI, 686; Cottin, *Rev. méd. de la Suisse Rom.*, 1914, XXXIV, 715; Bigelow, *Cleveland Med. Jour.*, 1915, 106; Pollock, *Trans. Chic. Path. Soc.*, 1916, X, 43; Larkin and Cornwall, *Jour. Lab. and Clin. Med.*, 1910, IV, 352; Herrick and Dannenberg, *Jour. A. M. A.*, 1919, LXXIII, 1321. Wynn (*Jour. Lab. and Clin. Med.*, 1922, VII, 273) points out that the cells may be safely counted if thoroughly mixed at any time up to at least 15 hours in the absence of macroscopic pellicle, sediment or web. If the fluids are not clear or become cloudy the results of counts at different periods vary.

² Nobécourt and Peyre, *Presse Méd.*, 1916, XXIV, 461, show that the protein content of the spinal fluid is persistently high as long as the infection persists. See Tashiro and Levinson, *Jour. Infect. Dis.*, 1917, XXI, 571.

³ See Frost, *Pub. Health Rep.*, 1912, XXVII, 97; Broers and Smit, *Nederl. Tijdschr. v.*

Bonome, while Jaeger and Heubner describe a diplococcus which is Gram-positive and may be confused with the unusual types of Weichselbaum's meningococcus.¹

Recently Flexner has succeeded in preparing an antimeningococcic serum which appears to have remarkable results in controlling this hitherto unmanageable disease. In using this serum, the injections must be made directly into the spinal cavity.² More or less frequent injections of the serum and examinations of the lumbar fluid are made and the influence of the serum estimated by bacteriological and cytological examinations. In the cases of mixed cerebral infection, in which the meningococcus is associated with the pneumococcus, streptococcus, typhoid bacillus, staphylococcus, and other organisms, this serum does not seem to have as much influence as in the pure meningococcus infections.³

It is to be remembered that a purulent meningitis may be secondary to infection with practically all of the pus-forming organisms found within the system. It is, therefore, essential that any infection showing meningeal symptoms should be investigated by an examination of the cerebrospinal fluid.

In sleeping sickness, a study of the cerebrospinal fluid frequently reveals the presence of the trypanosoma Gambiense. These parasites are not found in all cases, but when present usually furnish a grave prognosis.

Recent investigation of the cerebrospinal fluid, applying Wassermann's serum reaction for syphilis, has shown that in the large majority of nervous cases of syphilitic origin a positive reaction is obtainable.

Tubercular Meningitis.

The fluid in such cases is usually clear, but may be slightly opalescent. The cellular elements are largely mononuclear while a few red cells may be present. If tubercle bacilli cannot be found after careful search, the presence of a lymphocytosis along with an increased protein-content will be at least suggestive, while animal inoculation or the tuberculin test will clear up the diagnosis in most cases. (See Lucas.⁴)

Geneesk., 1915, II, 1175; also, Hort, Lakin and Benians (Brit. Med. Jour., 1915, I, 541), Foster (Ibid., 543) and Donaldson (Lancet, 1915, I, 1333), who suggest that the meningococcus is only a phase in the life history of the true infective agent. For studies of the question of meningococcus carriers see Reprint 413. 1917 from the Public Health Reports, U. S. P. H. S.; Alfaro, Arch. Latino-Amer. de Pediat., 1920, XIV, 28; Ponder, Brit. Med. Jour., 1920, II, 427; Glover, Ibid., 428; Dickson, Ibid., 430; Lister, Med. Jour. South Africa, 1921, XVI, 228.

¹ See Forbes (Brit. Med. Jour., 1920, II, 430; Lancet, 1920, II, 600) who reports a case of meningitis due to the *diplococcus crassus* an organism which is probably identical with the meningococcus of Jaeger. Cot and Robert (Paris Méd., 1921, VI, 318) describe a case of meningitis traceable to the micrococcus catarrhalis.

² See Dubois, Jour. Am. Med. Assn., 1913, LX, 820; also, Wollstein, Jour. Exper. Med., 1914, XX, 201; Dubois and Neal, Am. Jour. Dis. Child., 1915, IX, 1.

³ Collignon and Pilod (Presse méd., 1911, XIX, 732) advocate the following test to differentiate meningococcic from other types of meningitis. To a few c.c. (50 drops) of centrifuged spinal fluid add one to four drops of antimeningococcus serum and incubate the mixture (along with a control tube containing only spinal fluid) at 56°C. for 12 hours. If the serum and spinal fluid be homologous, a definite precipitation will be observed. This test appears to be specific.

⁴ Am. Jour. Dis. Child., 1911, I, 230; also, Mandelbaum, Deutsch. Arch. f. klin. Med., 1913, CXIII, 92; Roby, Jour. Am. Med. Assn., 1915, LXV, 1027; Kasahara, Am. Jour. Dis. Child., 1917, XIII, 141; Cooke, Jour. Am. Med. Assoc., 1922, LXXVIII, 430.

Acute Anterior Poliomyelitis.

This disease has been definitely established as infectious through the work of Flexner and his associates. The causative agent was known to belong to the ultra-microscopic type and to pass the Berkefeld filter. In other words it is a filterable virus, the point of ingress and egress of which is the nasal and pharyngeal mucosa. This virus is transmitted by means of the biting stable-fly (*Stomoxys calcitrans*) and probably, also, by the common fly and bedbug.¹

Cultivation of this organism has been accomplished by Flexner and Noguchi.² The culture medium consists essentially of sterile human ascitic fluid to which has been added a fragment of sterile fresh kidney tissue of the normal rabbit. Solid media is made by the addition of 2 per cent. agar to above. The exclusion of oxygen is necessary for the initial culture, but it suffices to cover the media with a deep layer of sterile paraffin oil. Films prepared from the lower layers of the fluid cultures are made, are air-dried and fixed in methyl alcohol for one hour. Wash in distilled water and immerse in Giemsa's solution (1 drop of stain to 1 c.c. of distilled water) for two to twelve hours. Examination reveals minute globoid bodies arranged in pairs or short chains, or in small aggregated masses. These bodies are stained violet and average about 0.2 micron in diameter. Their pathogenicity has been proven by inoculation of monkeys.

The changes in the spinal fluid, while not absolutely diagnostic are suggestive. The amount of fluid usually increases early in the disease, this increase being associated with a relative increase in the mononuclear types. These cells may first be of the small lymphocytic variety but they soon changed to the large mononuclear form. As the disease progresses, the cells diminish in number, the polymorphonuclear forms assuming the ascendancy. The total protein is increased in amount (as detected by the Noguchi or Ross-Jones method), while fibrin formation appears to be an early sign, although this may soon disappear.³

¹ Rosenau, Discussion in 15th Cong. on Hyg. and Demog., Abs. in Jour. Am. Med. Assn., 1912, LIX, 1314; Anderson and Frost, Pub. Health Rep., 1912, XXVII, 1733; Howard and Clark, Jour. Exper. Med., 1912, XVI, 850; Mitzmain, Pub. Health Rep., 1913, XXVIII, 345; Lucas and Osgood, Jour. Am. Med. Assn., 1913, LX, 1611; Rosenau, Ibid., 1612; Sawyer and Herms, Ibid., 1913, LXI, 461.

² Jour. Exper. Med., 1913, XVIII, 461. See, also, Amoss, Ibid., 1914, XIX, 212.

³ See "A Clinical Study of Acute Poliomyelitis" by Peabody, Draper and Dochez, published by the Rockefeller Institute in 1912; Flexner, Science, 1912, XXXVI, 685; Fraser, Jour. Exper. Med., 1913, XVIII, 242; Kling and Levaditi, Ann. de l'Inst. Pasteur, 1913, XXVII, 718; Cassel, Deutsch. med. Wchnschr., 1913, XXXIX, 2507; Lust and Rosenberg, Münch. med. Wchnschr., 1914, LXI, 121; Flexner and Amoss, Jour. Exper. Med., 1914, XIX, 411; Ibid., 1914, XX, 240; Ibid., 1915, XXI, 509; Flexner, Noguchi and Amoss, Ibid., 91; Flexner, Bull. Johns Hopkins Hosp., 1915, XXVI, 180; Am. Jour. Dis. Child., 1915, IX, 353. See, also, Morse, Boston Med. and Surg. Jour., 1914, CLXX, 373; Corbus, Jour. Am. Med. Assn., 1914, LXIII, 550; Neisser, Berl. klin. Wchnschr., 1915, LII, 486; Abramson, Am. Jour. Dis. Child., 1915, X, 344; Neal and Dubois, Am. Jour. Med. Sc., 1916, CLII, 313; Flexner, Ibid., 1917, CLIII, 157; Kolmer, Arch. Pediat., 1917, XXXIV, 413; Neal, N. Y. Med. Jour., 1916, CIV, 167; Klein, Ibid., 219; Flexner, Jour. A. M. A., 1916, LXVII, 279; Hoyne and Cepelka, Ibid., 666; Rosenow, Towne, and Wheeler, Ibid., 1202; Nuzum and Herzog, Ibid., 1205; Nuzum, Ibid., 1437; Mathers and Tunnickliff, Ibid., 1915; Nuzum, Ibid., 1917, LXVIII, 24; Abramson, Ibid., 546; Gauss, Ibid., 779; Greeley, Boston Med. and Surg. Jour., 1917, CLXXVI, 540; Jour. Lab. and Clin. Med., 1917, III, 671; Kolmer, Freese, Matsumami and Meine, Am. Jour. Med. Sc., 1917, CLIV, 720; Rosenow and Towne, Jour. Med. Res., 1917, XXXVI, 175; Flexner and Amoss, Jour. Exper. Med., 1917, XXV, 499, 525 and 539; Amoss and Taylor, Ibid., 507; Amoss, Ibid., 545; Bull, Ibid., 557; Amoss and Chesney, Ibid., 581; Kolmer, Brown and Freese, Ibid., 789; Mathers,

Cerebrospinal Syphilis.

Although such cases do not show pathognomonic findings in the cerebrospinal fluid, yet certain factors are of importance in diagnosis. A lymphocytosis is usually observed together with a marked increase in the globulin content, which is estimated as follows:¹ The technic and value of the Wassermann test have been discussed in detail in the section on Blood.

Noguchi's Butyric Acid Test.

One part (0.1 or 0.2 c.c.) of spinal fluid is mixed with 5 parts (0.5 c.c.) of a 10 per cent. butyric acid solution in physiologic salt solution. This mixture is heated to boiling and immediately 1 part (0.1 c.c.) of normal (4 per cent.) sodium hydrate solution is added and the mixture again boiled for a few seconds. The presence of an increased content of protein in the fluid is indicated by the appearance of a granular or flocculent precipitate which gradually settles under a clear supernatant liquid. This precipitate appears within a few minutes in a specimen containing a considerable increase in protein, while two hours may be required to obtain a distinct reaction in specimens weaker in protein. May² has called attention to the advisability of using larger portions of the fluid, preserving the same proportions of reagents as used by Noguchi.

This reaction appears regularly in the cerebrospinal fluid of patients with syphilitic and parasyphilitic affections and also in all cases of inflammation of the meninges caused by such organisms as the meningococcus, pneumococcus, influenza bacillus, tubercle bacillus, etc. These latter cases are, however, easily differentiated from the syphilitic affections. Normal fluid gives a turbidity but the granular precipitate does not occur at all or only after many hours.

Although this reaction is given in many non-syphilitic conditions, it has a certain value. It is not specific and, when present, does not necessarily indicate a syphilitic infection. On the other hand, it can be employed to establish or confirm a deduction based upon the clinical history and the results of the Wassermann reaction and cytodagnosis, thus becoming of great indirect diagnostic value. A syphilitic infection is, however, practically excluded by

Trans. Chic. Path. Soc., 1917, X, 145; Jour. Infect. Dis., 1917, XX, 113; Hektoen, Mathers, and Jackson, *Ibid.*, 1918, XXII, 89; Heist, Solis-Cohen, and Kolmer, *Ibid.*, 1909; Solis-Cohen and Heist, *Ibid.*, 175 and 182; Rosenow and Wheeler, *Ibid.*, 281; Solis-Cohen and Heist, *Ibid.*, 175 and 182; Rosenow and Wheeler, *Ibid.*, 281; Rosenow and Gray, *Ibid.*, 345; Rosenow, Towne, and von Hess, *Ibid.*, 313; McCann, Jour. Exper. Med., 1918, XXVII, 31; Smillie, *Ibid.*, 319; Tsen, *Ibid.*, 1918, XXVIII, 269; Schultz and Dunnenberg, Arch. Int. Med., 1919, XXIII, 309; Flexner and Amoss, Jour. Exper. Med., 1919, XXIX, 379; *Ibid.*, 1920, XXXI, 123.

¹ See Kaplan and Casamajor, Arch. Int. Med., 1912, IX, 262; also, Ball, Jour. Am. Med. Assn., 1912, LIX, 1272. Ellis (Jour. Exper. Med., 1913, XVIII, 162) asserts that no syphilitic patient should be regarded as cured without a complete negative examination of the cerebrospinal fluid. Miller, Southern Med. Jour., 1915, VIII, 940; Collins, Am. Jour. Med. Sc., 1916, CLI, 222; Reasoner, Jour. A. M. A., 1916, LXXVI, 1917; Levin, N. Y. Med. Jour., 1916, CIV, 212; With, Hospitalstid., 1916, LVIII, 1251, 1275 and 1311; Wile, Am. Jour. Syph., 1917, I, 84; Babonneix and Javiller, Bull. et mem. soc. de Hop. de Paris, 1918, XXXIV, 15; McIver, Jour. A. M. A., 1919, LXXIII, 1765; Larkin and Cornwall, Am. Jour. Syph., 1919, III, 76; Moore, Jour. Am. Med. Assoc., 1921, LXXVI, 769; Solomon and Klauder, *Ibid.*, LXXVII, 1701; Goedhart, Nederl. Tijdschr., 1921, II, 2190.

² Arch. Int. Med., 1911, VIII, 183.

a negative reaction. In this respect this test has advantages over the Wassermann reaction, in which a negative result is not always reliable as indicating absence of syphilitic infection.¹

Noguchi's New Method.

This method² is a flocculation test for the proteins of the cerebrospinal fluid, which is simpler than the butyric acid test and appears to offer promise of value as a diagnostic test. It is not altogether based on the direct precipitation of the proteins but is due to a concomitant flocculation of certain lipoids which the reagent contains. The volume of the precipitate produced is, therefore, more copious than that brought about by the direct precipitation methods.

Preparation of Solution 1.

This is a stock solution of acetone-insoluble lipoids. Beef heart is ground in a sausage machine and then completely dried by a fan over a heater. 100 grams of the dried substance are extracted with 1 liter of acetone for 5 days at room temperature, with daily shakings. The acetone is then discarded and the mass of solids freed from acetone by evaporation and then extracted with 1 liter of absolute alcohol, for 5 days at room temperature. The golden yellow alcoholic extract, which contains the acetone-insoluble tissue lipoids is separated from the dried muscle and tested for its suitability as the reagent. The criterion of usefulness is the transparency of a mixture of the alcoholic extract and Solution 2 in a ratio of 1:9. If a marked opalescence bordering on opacity is produced, the extract is unsuitable.

Preparation of Solution 2.

One and five-tenth grams of acid potassium phosphate (KH_2PO_4) and 4 grams of sodium chlorid are dissolved in 990 c.c. of distilled water containing 0.5 c.c. of glacial acetic acid. Finally, 10 c.c. of a saturated solution (approximately 3.5 per cent.) of picric acid in absolute alcohol is added. If the solution is not to be kept in the refrigerator, it is desirable, in order to prevent any fungus growth, to make it up in 10 times the strength in which it is to be used, that is to dissolve the salts in 90 c.c. of distilled water containing 0.5 c.c. of glacial acetic acid and add 10 c.c. of the picric acid solution. At the time of use, 1 part of this latter solution should be mixed with 8 parts of distilled water and 1 part of the lipoidal solution added.

Preparation of Reagent.

To 9 parts of solution 2 is added gradually, mixing by gentle agitation, 1 part of the alcoholic lipoidal extract (solution 1), the resulting mixture being faintly opalescent, almost transparent. The mixture in this form remains unchanged for a period of several weeks, but as the two solutions may be preserved separately for an indefinite period, the reagent can be made fresh as needed.

¹ Nichols and Hough (Jour. Am. Med. Assn., 1913, LX, 108) report the demonstration of the spirochete *Treponema pallidum* in the cerebrospinal fluid and the successful inoculation of a rabbit therefrom.

² Jour. Am. Med. Assoc., 1921, LXXVI, 632.

Technic.

Into a small test tube, such as are used for the Wassermann test, is measured 0.1 c.c. of the cerebrospinal fluid and 1 c.c. of the reagent is then added. Normal fluids remain perfectly clear or become only faintly opalescent, while a dense general turbidity is produced in all specimens containing an increased amount of globulin or albumin. In fluids from bacterial meningitis, the flocculation is dense and copious, followed by complete or partial precipitation of the granular flocculi within about an hour at room temperature. Specimens from general paresis and tabes give a dense flocculation, somewhat less copious than those from acute inflammations, the granular flocculi settling to the bottom of the tube within a few hours. The reaction is rapid, the maximum opacity being reached within a few minutes; the granulation and subsequent sedimentation of the flocculi, however, require a longer time. The reaction occurs at any temperature from that of the ice box to that of the incubator, but room temperature has been found most satisfactory.

Nonne's Test.

This test,¹ like the above, is for the detection of an increase of the protein material in the spinal fluid. It is, however, not limited to syphilitic conditions, although it is widely used for such cases. Nonne has advanced two steps which he styles "phases."

Phase I.

To a small portion of the clear cerebrospinal fluid add an equal volume of a hot saturated solution of ammonium sulphate. A trace of opalescence or a distinct turbidity, usually appearing within three minutes, indicates a positive reaction. Normal fluids never show any appreciable change with this test, while pathologic types always react promptly. The test is positive in all infections of the nervous system and is of special interest in general paralysis, tabes and obscure cerebrospinal syphilis. Although not absolutely differential, it is, yet, of great importance, as a negative test excludes syphilis and parasymphilis.

Phase II.

In case no turbidity is observed in Phase I, add a drop or two of acetic acid to the above mixture. A distinct turbidity appears at once. This phase is given, not infrequently, with normal fluids so that little value is attached to it.

Ross-Jones Test.

This² is a modification of Phase I of Nonne and is, in my opinion, somewhat more delicate. Two c.c. of a saturated solution of ammonium sulphate are placed in a test-tube and 1 c.c. of the cerebrospinal fluid is gently run on to the surface so as to form a contact layer. A clear-cut, thin, grayish-white ring is observed at the junction of the liquids if the reaction be positive. This

¹ Arch. f. Psychiat. u. Nervenkrankh., 1907, XLIII, 433; Münch. med. Wehnschr., 1907, LIV, 2117.

² Brit. Med. Jour., 1909, I, 1111.

ring should appear within three minutes. The value of the test is the same as that above.

Lange's Colloidal-gold Test.

As neither the Nonne nor Ross-Jones tests exclude inflammatory processes in the brain or cord, a more differential test is to be desired. This seems to have been found in Lange's test. It is based on the fact that protein material precipitates colloidal gold at a dilution which is constant for definite proteins. If the protein be in less concentration a protective power over the colloidal particles is exerted and no precipitation occurs. In the case of spinal fluid, the protein present in different pathologic conditions shows a different precipitating point in syphilitic and non-syphilitic disorders. This test is, therefore, much more differential than are the preceding ones given.

Preparation of the Colloid Solution.

The preparation of a satisfactory solution for this test is a matter of some difficulty, as evidenced by the numerous methods published. The technic which the writer prefers is the one of Gettler and Jackson¹ which is very similar to that of Miller, Brush, Hammers and Felton published some years previously.

Into a clean 1.5 liter flask place 1 liter of specially distilled water. (For preparation of the water proceed as follows: To several liters of water in a large glass flask, add a few crystals of potassium permanganate and distil, rejecting the first 300 c.c.). To the water in the liter flask add 10 c.c. of 1 per cent. aqueous solution of gold chlorid, 7 c.c. of 2 per cent. potassium carbonate solution, and 0.5 c.c. of 1 per cent. aqueous solution of oxalic acid. Heat this mixture to the boiling point, and at this temperature remove the flask from the flame, holding it by means of a towel, and shake the contents vigorously. While the solution is still in motion, add quickly from 0.2 to 0.3 c.c. of formaldehyd (formalin, 40 per cent.) and at once shake the flask and contents thoroughly for from $\frac{1}{2}$ to 1 minute. After from 3 to 4 minutes the color usually commences to develop. If, however, there should be no indication of color, the solution must again be shaken well and, while still in motion, an additional 0.1 to 0.2 c.c. formalin added; this addition almost invariably produces the desired change. At no time during the process should the agitation be stopped. The color should develop rapidly to a deep red. If, however, there is a delay in the appearance of color, the mixture should be allowed to stand, and in a minute or two the color will start to develop; at this instant the solution should again be thoroughly shaken until the color reaches a deep red shade. In addition to having the proper shade, a deep red clear to both direct and transmitted light and occasionally with a slight golden shimmer, this solution must remain unchanged when run with a known normal spinal fluid, should give a typical curve with a known positive fluid, and 5 c.c. of it should be completely precipitated in one hour by 1.7 c.c. of 1 per cent. sodium chlorid solution, and must be neutral to a 1 per cent. solution of alizarin red in 50 per cent. alcohol.

¹ Arch. Neurol. & Psychiat., 1921, VI, 71.

Technic.

Place in a rack a series of twelve test-tubes. In the first, place with a pipet 1.8 c.c. of 0.4 per cent. freshly made sodium chlorid solution. In the other eleven tubes place 1 c.c. of the chlorid solution. To the first tube add 0.2 c.c. of spinal fluid, which must be free from blood. The dilution in tube 1 is, therefore, 1 to 10. Mix this thoroughly and place 1 c.c. of this first dilution in the second tube. This dilution is 1 to 20. Proceed in the same way with the remaining tubes, getting dilutions up to 1 to 20,440.

After the dilutions are prepared, add to each tube 5 c.c. of the colloidal solution and let the tubes stand for 24 hours at room temperature. The reading of the test is as follows: The original tube showed a medium magenta shade. As the precipitation of the gold occurs, the color of the tubes constantly manifests a bluer tinge and finally becomes clear and colorless. The dilution at which the greatest precipitation occurs is noted and the results at various dilutions expressed by the + or - sign. Thus - indicates no precipitation; +, a red with beginning blue tinge; ++, red-blue and blue-red shades; +++, violet and dark blue colorations; +++, light blue color; +++++, complete precipitation with a clear and colorless solution.¹

In syphilitic and parasyphilitic cases the tendency of the precipitation is constantly toward the left of the series, that is toward the 1 to 10 dilution. The maximum effect in such conditions is 1 to 40 or 1 to 80. It never continues past 1 to 640 and infrequently past 1 to 160. In non-syphilitic cases the tendency is toward the right of the series, that is toward the 1 to 20,000

¹ Berl. klin. Wchnschr., 1912, XLIX, 897; Ztschr. f. Chemotherap., 1912, I, 44. See, also Grulee and Moody, Jour. Am. Med. Assn., 1913, LXI, 13; Eicke, Münch. med. Wchnschr., 1913, LX, 2713; Kaplan and McClelland, Jour. Am. Med. Assn., 1914, LXII, 511; Miller and Levy, Bull. Johns Hopkins Hosp., 1914, XXV, 133; Kafka and Rautenberg, Ztschr. f. d. ges. Neurol. u. Psychiat., 1914, XXII, 353; Lee and Hinton, Am. Jour. Med. Sc., 1914, CXLVIII, 33; Debenedetti, Rif. Med., 1914, XXX, 906; Brock, Ill. Med. Jour., 1914, XXVI, 422; de Crinis and Frank, Münch. med. Wchnschr., 1914, LXI, 1216; Kafka, Ibid., 1915, LXII, 105; Solomon and Koefod, Boston Med. and Surg. Jour., 1914, CLXXI, 886; Solomon and Welles, Ibid., 1915, CLXXII, 398 and 625; Weston, Darling and Newcomb, Am. Jour. Insan., 1915, LXXI, 835; Grulee and Moody, Am. Jour. Dis. Child., 1915, IX, 17; Barnes and Ives, Interstate Med. Jour., 1915, XXII, 792; Swalm and Mann, New York Med. Jour., 1915, CI, 719; Rubenstone and Schwartz, Ibid., 1273; Busquet, Bull. l'Acad. Med., 1915, LXXIV, 183; Miller, Brush, Hammers, and Felton, Bull. Johns Hopk. Hosp., 1915, XXVI, 391; Solomon, Koefod, and Welles, Boston Med. and Surg. Jour., 1915, CLXXIII, 956; Solomon and Welles, Ibid., 1916, CLXXIV, 50; Ramsay and Fidler, Can. Med. Assoc. Jour., 1916, VI, 685; Oetiker, Ztschr. f. klin. Med., 1916, LXXXII, 235; Williams and Burdick, Colo. Med., 1916, XIII, 122; Weston, Jour. Med. Res., 1916, XXXIV, 107; Ibid., 1917, XXXV, 367; Trimble, Jour. Lab. and Clin. Med., 1917, II, 199; Jeans and Johnston, Am. Jour. Dis. Child., 1917, XIII, 239; Felton and Maxey, Jour. A. M. A., 1917, LXVIII, 752; Hammes, Am. Jour. Med. Sc., 1917, CLIV, 625; Vogel, Arch. Int. Med., 1918, XXII, 406; Rawlings, Arch. Neurol. & Psychiat., 1919, II, 180; Solomon, Ibid., III, 49; Iida and Tominaga, Mitt. a. d. Med. Fak. d. Univ. Tokyo, 1919, XXI, 217; Prunnell, Rev. Med. del Uruguay, 1919, XXII, 620; Eicke, Münch. med. Wchn., 1919, LXVI, 1049; Cruickshank, Brit. Jour. Exp. Path., 1920, I, 71; Jour. Path. & Bact., 1920, XXIII, 232; Kellert, Am. Jour. Med. Sc., 1920, CLIX, 257; Nixon, Minn. Med., 1920, III, 186; Warwick, Ibid., 188; Warwick and Nixon, Arch. Int. Med., 1920, XXV, 119; Weston, Am. Jour. Insan., 1920, LXXVI, 393; McDonagh, Lancet, 1920, II, 991; Davis and Kraus, Am. Jour. Med. Sc., 1921, CLXI, 109; Haguenau, Ann. de Med., 1921, IX, 430; Terashima, Jap. Med. World, 1921, I, 21; Warwick, Arch. Int. Med., 1921, XXVII, 238; Fischer, Ztschr. f. ges. exp. Med., 1921, XIV, 60; Mazza, Mey and Nino, Rev. de la Assoc. Med. Argentina, 1921, XXXV, 532; Sordelli and Renella, Ibid., 535; Keidel and Moore, Arch. Neurol. & Psychiat., 1921, VI, 163; Howell, Ibid., 1922, VII, 229; Regan and Cheney, Am. Jour. Dis. Child., 1922, XXIII, 107; Adams and Scott, Jour. Path. & Bact., 1922, XXV, 142.

dilution, the maximum being observed at some point above 1 to 80. Tuberculous meningitis shows the most intense reaction at 1 to 160 or 1 to 320, while suppurative meningitis gives most marked reactions at 1 to 1,200 and up. The typical reactions may be read thus, giving numerals to the strengths of reaction in the consecutive tubes as noted by the change of color from red, through red-blue, violet, blue, gray, to colorless as 0, 1, 2, 3, 4, and 5. Normal 0000000000; syphilitic, 0123331000; non-luetic (inflammatory), 0001234210; parietic, 5555542100. This reaction promises to be of great value, especially in congenital syphilis.¹

¹ Other methods for the detection of increased amounts of organic matter and protein in the cerebrospinal fluid have been introduced, but none of these have any advantage over those mentioned above. Among these methods we find Pandey (Neurol. Zentralbl. 1910, XXXIX 915) method of precipitation with phenol (1:16); the Permanganate Reduction Index of Mayerhofer (Wien. klin. Wchnschr., 1910, XXIII, 651); in this connection see Hoffman and Schwartz, Arch. Int. Med., 1916, XVII, 293; the Permanganate Test of Boveri (Münch. med. Wchnschr., 1914, LXI, 1215; see, also, Rubenstone, N. Y. Med. Jour. 1915, CII, 1052; Lowrey, Boston Med. and Surg. Jour., 1917, CLXXVII, 115; Genoese, Policlinico, 1919, XXVI, 97; Camp. Am. Jour. Syph., 1920, IV, 301; Guillaïn & Libert, Ann. de Méd., 1921, IX, 271; Boveri, Policlin., 1921, XXXVII, 430; the Mastic Test of Emanuel (Berl. klin. Wchnschr., 1915, LII, 792; see, also, Langdon, Jour. Lab. and Clin. Med., 1918, III, 376; Stanton, Arch. Neurol. & Psych., 1920, IV, 301; Kafka, Deut. med. Wchn., 1921, XLVII, 1422; the precipitation of protein with sulphosalicylic acid as advocated by Pfeiffer, Med. Record, 1916, LXXXIX, 66; and elaborated by Kirchberg, Deutsch. med. Wchnschr., 1918, XLIV, 657; Denis and Ayer, Arch. Int. Med., 1920, XXVI, 436; Ayer and Foster, Jour. Am. Med. Assoc., 1921, LXXVII, 365, and, recently the Lead Peroxid Reaction of Steinfeld, Jour. Lab. and Clin. Med., 1919, IV, 445; and the precipitation of the protein with an acidified solution of potassium dihydrogen phosphate, as advocated by Amoss, Jour. A. M. A., 1919, LXXII, 1289.

COLLOIDAL BENZOIN REACTION

Guillaïn, Laroche and Lechelle (C. R., Soc. de Biol., 1920, LXXXIII, 1977; Bull. Soc. Med. des Hop., 1920, XLIV, 1209; Progress Med., 1920, XXXV, 518; Bull. Soc. Med. des Hop., 1921, XLV, 355; Presse Med., 1921, XXIX, 773; "La Reaction du Benjoin Colloidal," Paris, 1922) have introduced a test, which depends upon the flocculation of benzoïn from a colloidal solution in the presence of an excess of protein in the cerebrospinal fluid. This test seems to parallel the Wassermann test to as great extent as does the colloidal gold test and has the advantage that the test solution is much more easily prepared. The writer has used this test in his laboratory ever since its publication and has checked it both with the Lange and Wassermann reactions. If the benzoïn solution is prepared from the amygdaloid benzoïn, as advocated by the originators of the test, the results may be relied upon, but if the usual powdered benzoïn of the drug houses be employed the results are far from certain and reliable. This test, known as the *Colloidal Benzoïn Reaction* is as follows: Two solutions are prepared, (1) a solution of C. P. sodium chlorid in water in the strength of 1:10,000; and (2) a solution containing a suspension of benzoïn resin in alcohol. The first solution is best made by diluting a stronger solution to the proper degree, as the weighing of small quantities of salt is not always accurate. The benzoïn solution is prepared by treating 1 gram of the natural resin of benzoïn, which is ground into a fine powder by the worker, with 10 c.c. of absolute alcohol (as stated above the powdered benzoïn of commerce should not be used). This mixture is thoroughly shaken and allowed to stand for 48 hours, at the end of which time the clear alcoholic solution is decanted and kept in dark bottles. One-tenth c.c. of this alcoholic solution is then slowly added to 20 c.c. of double distilled water which has been heated to 35°C. These suspensions should be freshly made at least every second day. The technic is as follows: Set up a series of 16 small test tubes, such as are used in the Wassermann test and to the first tube add 0.25 c.c. of the dilute sodium chlorid solution; to the second tube 0.50 of this salt solution; and to the third tube 1.5 c.c. In the remaining 13 tubes place 1 c.c. of the salt solution. To the first of these tubes is then added 0.75 c.c. of the spinal fluid; to the second and third tubes 0.5 c.c. each of spinal fluid. Then 1 c.c. of the dilution in the third tube is transferred to tube 4; then 1 c.c. of the mixture in tube 4 transferred to tube 5 and so on to tube 15 from which 1 c.c. is removed and discarded after the mixture has been made with the dilution from tube 14. Tube 16 acts as the control. The dilution in tube is $\frac{3}{4}$; in tube 2 is $\frac{1}{2}$; in tube 3 is $\frac{1}{4}$, the successive dilutions increasing up to $\frac{1}{16384}$ in tube 15. To each of the 16 tubes is then added 1 c.c. of the benzoïn solution

and the mixtures set aside at room temperature for 6 to 12 hours. In the positive tubes the precipitation is absolute, the liquid becoming perfectly clear and limpid and the resin being completely settled at the bottom of the tube. In the negative tubes the turbid appearance persists without any precipitate, the tubes being exactly the same as the control. Between these extremes there may appear a reaction, which is called subpositive, the mixture still being turbid but presenting a more or less copious residue at the bottom of the tube. These reactions are designated as follows: 0, the negative; 1, the subpositive; and 2, the positive test. In reading the test these figures may be used, as in the case of the Lange reaction. Normally there is no reaction in any of the tubes, except occasionally in 1 or 2 of the later tubes. In general paresis the precipitation is complete in tubes 1 to 5, 6, 8, 9, 10, even up to 12 of 13 that is, a reaction 222221222100000. In active tabes the reaction is often as marked as in general paresis but, occasionally, one finds the positive reactions only up to tube 6 or 7. The same is true of any diffuse syphilitic process. The flocculation practically always begins in tube one, but occasionally a case may be found in which this does not occur till tube 2. Flocculation in the first 5 tubes, and from that up to variable limits, constitutes the characteristic "syphilitic zone," the results paralleling the Wassermann test in about the same degree as the Lange test. Flocculation above this zone is evidence of non-specific disease. It is to be remembered that this test can not be applied to purulent, turbid or bloody spinal fluids, as false reactions will almost invariably arise. See Duhot and Crampon, *C. R., Soc. de Biol.*, 1920, LXXXIII, 1421; Huber, *Ibid.*, LXXXIV, 496; Pautat, *Ibid.*, 503; Benard, 1921, LXXXV, 210; Targowla, *Ann. de Méd.*, 1921, X, 275; Gastinel and Jacob, *Bull. Med.*, 1921, XXXV, 315; Ferrare, *Policlinico*, 1922, XXIX, 77; Terzani, *Riv. Crit. de Clin. Med.*, 1922, XXIII, 1 and 13; Warnock, *Jour. Lab. & Clin. Med.*, 1922, VII, 400.

CHAPTER X

SECRETION OF THE MAMMARY GLANDS

I. GENERAL CONSIDERATIONS

The normal secretion of milk takes place in the mammary glands of the female after delivery. It is true that a small quantity of milk may be secreted by the new-born of both sexes for a few days after birth. Moreover, cases have been reported in which the adult male secreted sufficient milk to act as a wet-nurse, but these must be regarded as cases of extreme rarity.

During the course of a normal pregnancy a small amount of a thin, yellowish fluid may be expressed from the mammary glands, but as a rule the first real secretion is observed following delivery of the child.¹ This secretion is thin and watery, more or less translucent, and shows a distinct yellowish color.

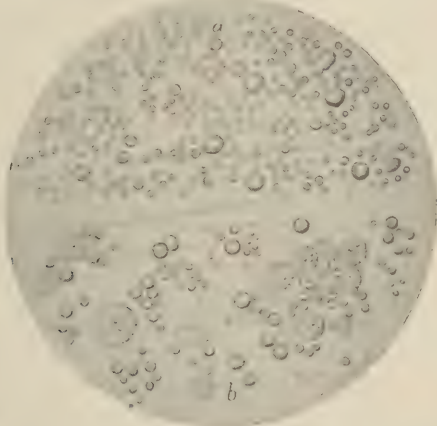


FIG. 162.—Normal Milk and Colostrum. (Hawk.)
a, Normal milk; b, colostrum.

Microscopic examination shows the presence of rather large cells in which are many fat granules and occasionally a distinct nucleus. This secretion, which is called *colostrum*,² continues for three to four days and is distinguishable from the later secretion by the fact that it contains relatively more salts than normal milk and, according to the usual statements, more sugar. In a series of determinations of breast milk collected during the fourth day after

¹ Luzzatti (Policlinico, 1920, XXVII, 1430) reports of case in which colostrum began to flow at the fourth month and continued until term, while Del Vecchio (Ibid., 1921, XXVIII, 262) cites his case of colostrorrhea from the 5th to 7th month.

² See Engel and Bauer, *Die Biochemie und Biologie des Kolostrums*, Wiesbaden, 1912, Bergmann; Benestad, *Monatsschr. f. Geburtsh. u. Gynäk.*, 1914, XL, 674; Holt, Courtney and Fales (*Am. Jour. Dis. Child.*, 1915, X, 220) indicate that in the colostrum period, woman's milk has high protein and high ash with rather low fat values; Hammett (*Jour. Biol. Chem.*, 1917, XXIX, 381) shows that fat and lactose were increased in amount and protein diminished during the first eleven days after parturition. Lewis and Wells (*Jour. Am. Med. Assoc.*, 1922, LXXVIII, 863) believe the function of the colostrum is to supply euglobulin to the child in the early days of birth. Howe (*Jour. Biol. Chem.*, 1922, LII, 51) has introduced a method for the differential precipitation of the proteins of colostrum.

delivery, the writer could not show any marked increase in the sugar in all cases, but in three out of eight cases examined a percentage higher than eight of lactose was obtained.

It is probable that the function of the colostrum is to supply euglobulin for a short period after birth and gradually to accustom the child to the taking of food by diminishing to a slight extent the elements, protein and fat, which are more apt to cause digestive disturbances than is the lactose.

The secretion of true milk begins about the fourth day and continues for a variable length of time. Marked variations are observed both in the quantity and the quality of this secretion in various women so that no hard and fast rule can be given as to the composition of normal mother's milk. While it is probably true that the normal woman should nurse her child during a large part of the first year, it is rare to find, especially in private practice, many such cases. Either the milk becomes scanty and loses in nutritive power or becomes excessive and consequently diluted. In either case the child is not receiving the most suitable nourishment, so that breast-feeding is abandoned under these circumstances. It seems to be a general rule, which is difficultly explicable, that the more highly socially developed the woman the less apt she is to nurse her child with any success. It is possible, and this seems to the writer the most probable explanation, that the child usually receives more or less constant attention from the physician, and variations in the breast milk are more frequently noticed than in the case of the poorer child who rarely has the advantage of medical attention unless more or less serious illness occurs.

II. PHYSICAL AND CHEMICAL PROPERTIES

Various figures have been given for the composition of human milk so that it is difficult to strike an average. So much depends upon the nourishment of the mother, upon the amount of exercise taken by her, and upon the general condition of the system that marked variations exist in the proportion of the chemical constituents. The following table, taking averages of examinations reported by various writers, may serve as one representing more nearly the normal condition.¹

Water,	87.24
Salts,	0.26
Protein,	1.50
Fat,	4.00
Lactose,	7.00

These figures are not as high as regards protein as those usually given, but are higher as far as the lactose is concerned. Although many writers have

¹ See Schloss, *Monatsschr. f. Kinderhkde.*, 1912, X, 499; also, Meigs and Marsh, *Jour. Biol. Chem.*, 1913, XVI, 147; Talbot, *Am. Jour. Dis. Child.*, 1914, VII, 445; Grulee and Caldwell, *Ibid.*, 1915, IX, 374; Hart and Humphrey, *Jour. Biol. Chem.*, 1915, XXI, 239; Bosworth and Van Slyke, *Jour. Biol. Chem.*, 1916, XXIV, 187; Hammett and McNeile, *Ibid.*, 1917, XXX, 145; Raimondi, *Le Nourrison*, 1919, IV, 269; Marfan, *Jour. physiol. et de path. Gen.*, 1920, XVIII, 985; Osborne and Lafayette, *Jour. Biol. Chem.*, 1920, XLI, 515; Sommer and Hart, *Ibid.*, 617; Denis, Sisson and Aldrich, *Ibid.*, 1922, L, 315; Hartwell, *Biochem. Jour.*, 1922, XVI, 78.

given the percentage of protein much higher than 1.5 per cent., the writer has never been able, in several hundred examinations of milk in his laboratory, to find many showing a protein percentage of two or more.

As it is frequently necessary to modify cow's milk so that it may more nearly approach mother's milk in composition, the writer inserts the following table for comparison.

Water,	87.25
Salts,	0.75
Protein,	3.50
Fat,	4.00
Lactose,	4.50

It will be seen that cow's milk shows a higher per cent. of protein and a lower per cent. of carbohydrate. It is necessary, therefore, that this be modified by diluting the milk so as to diminish the protein and by adding lactose to make up for the deficiency of carbohydrate. In the dilution the fat content will necessarily be lowered so that this may be remedied, as suggested by Backhaus, by the addition of cream. The writer must refer to works on pediatrics for the various methods of modifying cow's milk. It is to be remembered that no modification is equal to mother's milk. The casein of human milk forms a much finer clot with the gastric juice than does that of cow's milk so that the latter may not be well tolerated by the child. Moreover, some unknown principle present in human milk is accountable for a distinct biologic difference in these two types of nutritive material.

(1) **Appearance and Color.**

Normal human milk is a white fluid which usually has a slight bluish tinge except immediately after birth when the color may be distinctly yellowish from the presence of colostrum.¹ If the percentage of fat be relatively high the color will be more nearly a pure white with little of the blue tone.

(2) **Specific Gravity.**

The specific gravity of human milk varies between 1.028 and 1.034. An increase in the percentage of fat will usually lower the specific gravity while the protein and carbohydrates will increase it.

Cow's milk shows approximately the same specific gravity. If a low specific gravity is obtained it is evidence either of an increased percentage of fat or of dilution with water. If the percentage of fat be low the milk is unquestionably a watered one. If the milk tested shows a high specific gravity the chances are that most of the fat has been removed either by skimming or by centrifugation.

The determination of the specific gravity may be made by a special instrument known as Quevenne's lactodensimeter or by the use of the ordinary hydrometer used in urine work. As it is never of any clinical importance or even of any marked practical importance that the specific gravity should be absolutely accurately determined, the writer is accustomed to use the urinometer for such determinations. Corrections for variations in temperature

¹ Feer (Biochem. Ztschr., 1916, LXXII, 378) has reported a greenish coloration of the milk after eating calves liver.

are necessary only when great differences exist between the temperature of the room and the temperature at which the instrument is calibrated.

(3) Reaction.

Normal human milk as well as cow's milk shows an amphoteric reaction to litmus-paper and an acid reaction to phenolphthalein, cow's milk being somewhat more acid than mother's milk toward the latter indicator.¹

(4) Coagulation.

If milk be allowed to stand, the reaction gradually becomes more and more acid owing to the development of bacteria, especially of the *bacillus acidilactici*. When the degree of acidity reaches a certain point, casein separates first in the form of flocculi and later the entire fluid may coagulate to a jelly-like mass. This mass soon contracts and settles out leaving a slightly turbid fluid known as milk plasma or acid whey.² In order to inhibit the development of bacteria and prevent this coagulation, known as souring, certain preservatives are frequently added to market milk and should be capable of detection by the practitioner. These will be discussed in a later section.

Besides this type of coagulation of cow's milk a second form is observed which takes place under the influence of chymosin without any change in the reaction of the fluid. In this case the whey is sweet and contains practically all of the lactose originally present in the milk.³

(5) Total Solids.

Five to ten c.c. of the well-mixed milk are placed in a weighed platinum dish, evaporated to dryness on a water-bath, and dried to constant weight in the oven at 105°. The difference in weight between the platinum dish and its contents, on the one hand, and the platinum dish, on the other, gives the amount of total solids in the milk taken. A simple calculation will yield the percentage of total solids.

Under normal conditions the total solids of both human and cow's milk should average between 12 and 13 per cent. Variations in this figure are, of course, due to fluctuations in the various constituent elements.

(6) Ash.

The platinum dish containing the dried residue of the milk is heated over a direct flame until the residue is completely incinerated. This is then placed in the desiccator and dried to constant weight. The difference in weight between the dish and contents and the dish alone represents the amount of salts present in the milk originally taken.⁴

¹ See Clark, *Jour. Med. Research*, 1914, XXXI, 431, who shows that the P_H value for mother's milk is 7 to 7.2; Milroy, *Biochem. Jour.*, 1915, IX, 215; Van Slyke and Baker, *Jour. Biol. Chem.*, 1919, XL, 345 and 357; Schultz and Chandler, *Ibid.*, 1921, XLVI, 129.

² See Orla-Jensen, *Ztschr. f. physiol. Chem.*, 1914, XCIII, 283; Van Slyke and Bosworth, *Jour. Biol. Chem.*, 1916, XXIV, 191; Van Slyke and Baker, *Ibid.*, 1918, XXXV, 147; Sommer and Hart, *Ibid.*, 313.

³ See Sommer and Hart (*Jour. Biol. Chem.*, 1919, XL, 137) for a discussion of heat coagulation of milk; *Ibid.*, 1920, XLI, 617.

⁴ Bosworth, *Jour. Biol. Chem.*, 1915, XX, 707; Holt, Courtney and Fales, *Am. Jour. Dis. Child.*, 1915, X, 229; Meigs, Blatherwick and Cary, *Jour. Biol. Chem.*, 1919, XXXVII, 1; *Ibid.*, 1919, XL, 409; Holt, Courtney and Fales, *Am. Jour. Dis. Child.*, 1920, XIX, 97; Sisson and Denis, *Jour. Am. Med. Assoc.*, 1920, LXXV, 601; *Am. Jour. Dis. Child.*, 1921, XXI, 380.

(7) Protein.

The methods for the determination of protein are divided into those for estimation of the total protein material present and into those in which separate determinations are made of the casein, on the one hand, and albumin and globulin, on the other.

(a) Total Protein.**Method of Sebelien.**

Ten c.c. of milk are diluted with 90 c.c. of water, 5 c.c. of a saturated sodium chlorid solution, and 15 c.c. of Almén's tannic acid solution are added. The mixture is thoroughly stirred and the dense precipitate which forms is allowed to settle. The composition of Almén's tannic acid solution is as follows:

Tannic acid,	4 grams.
Acetic acid (25 per cent.),	8 c.c.
Alcohol (50 per cent.), qs., ad.,	200 c.c.

The precipitate, which consists of the total protein of the milk and a large portion of the fat carried down by the precipitate, is then filtered off through a fine filter and is washed with cold water. The filter-paper and its contents are then placed in a Kjeldahl flask and a nitrogen determination made as described under Urine. It is advisable to use 20 c.c. of sulphuric acid instead of the ten employed in the case of urine, as the mixture oxidizes much more rapidly under these conditions. If the nitrogen obtained in this determination be multiplied by 6.37, the result will be the protein in 10 c.c. of milk.

Method of Boggs.

For routine work this method is perhaps more advisable for the general practitioner than is the preceding, but it does not always give confirmatory results. It is based upon the fact that the total protein of milk is precipitated by phosphotungstic acid in hydrochloric acid solution, the amount of precipitate being measured in an Esbach tube. The reagent used has the following composition:

Phosphotungstic acid,	25 grams.
Concentrated hydrochloric acid,	25 c.c.
Distilled water, q.s., ad.,	250 c.c.

It has been found that the milk should be diluted with water before adding the reagent if the results are to be accurate. As a rule, a dilution of 1 to 10 for human milk and 1 to 20 for cow's milk suffices.

The diluted milk is poured into the Esbach tube¹ to the mark *U* and the reagent added to the mark *R*. The tube is then closed with a stopper and inverted several times thoroughly to mix the contents. It is then set

¹ If a Purdy centrifuge tube be filled with the diluted milk to the 10 c.c. mark and the reagent added to 15 c.c., the amount of protein may be determined quickly by centrifuging for 3 minutes and reading the percentage directly. The figures obtained agree quite closely with those of the gravimetric method.

aside for 24 hours when the percentage of protein in the milk is read off directly from the calibrations on the tube in case the dilution was 1 to 10, while with a dilution of 1 to 20 the figures are multiplied by 2.

This method, while convenient, is open to the objection that many factors may influence the depth to which a precipitate settles. Moreover, the Esbach tubes reading as high as 12 parts do not give as satisfactory results as those with readings from one to seven. Such being the case the method must be used more for clinical purposes than for scientific estimations.

(b) Casein.

Twenty c.c. of well-mixed milk are measured into a beaker and approximately 380 c.c. of water are added. The mixture is thoroughly stirred and very dilute acetic acid added *drop by drop* with constant stirring until a flocculent precipitate is observed. When this point is reached a stream of carbon dioxid is passed through the mixture for one-half hour, after which the vessel is allowed to stand until the next day. The above part of the technic is directly applicable to cow's milk. If human milk is being examined it is necessary to heat the vessel to 40°C. during the passage of the carbon dioxid.

After the mixture has stood overnight, it is filtered through a nitrogen-free filter and washed with water. The residue on the filter contains casein which is mixed with a portion of the fat present. The filter-paper and contents are then placed in a Kjeldahl flask and a nitrogen determination is made as previously described. Multiplication of the nitrogen value by 6.37 yields the amount of casein in the 20 c.c. of milk originally taken.¹

(c) Albumin and Globulin.

The filtrate from the above precipitation of casein contains the remainder of the protein material and the carbohydrate of the milk. This filtrate is placed in a porcelain dish and heated for a few minutes to the boiling-point. The protein material is coagulated and may be filtered through a nitrogen-free filter and washed several times with cold water. A nitrogen determination is then made and the value multiplied by 6.37 to obtain the amount of albumin and globulin present in the 20 c.c. of milk.²

The filtrate from this latter precipitation contains the lactose, which may then be determined by titration with Fehling's solution, as described under Urine. It is to be remembered that 10 c.c. of Fehling's solution are reduced by 0.0678 gram of lactose, and not by 0.05 as in the case of glucose.

¹ See Van Slyke and Bosworth, Jour. Biol. Chem., 1915, XX, 135; Hart and Humphrey, Jour. Biol. Chem., 1917, XXXI, 445; Osborne and Wakeman; Ibid., 1918, XXXIII, 7; Bosworth and Giblin, Ibid., XXXV, 115; Hart and Humphrey, Ibid., 367; Palmer and Scott, Ibid., 1919, XXXVII, 271; Van Slyke and Bosworth, Ibid., 285.

² See Emmett and Luros, Jour. Biol. Chem., 1919, XXXVIII, 257. Denis and Minot (Ibid., XXXVII, 353 and XXXVIII, 453) have introduced methods for the determination of the non-protein nitrogenous constituents of milk. (See, also, Kennedy, Jour. Am. Chem. Soc., 1919, XLI, 388; and Denis, Talbot, and Minot, Jour. Biol. Chem., 1919, XXXIX, 47). Osborne and Wakeman (Ibid., 1918, XXXIII, 243) have shown the presence of a further protein in cow's milk which is soluble in 50 to 70 per cent. alcohol but insoluble in absolute alcohol. They point out that this must be taken into consideration in methods having to do with the estimation of the non-protein nitrogenous constituents. Cary (Jour. Biol. Chem., 1920, XLIII, 477) believes the amino acids of the blood to be the precursors of the milk proteins. See, also, Nijikata, Ibid., 1922, LI, 165.

(8) Fat.

It is important in the determination of the fat content of milk that a thoroughly mixed specimen be examined.¹ As the fat tends to rise to the surface of the milk, the fluid should be poured from one vessel into another several times to insure thorough mixing and an immediate measurement made of the portion to be tested.

For clinical purposes as well as for examination of market milk the method of Babcock is to be recommended. For accurate results, however, this method is not to be advised.

Babcock's Method.

This method consists in the destruction of the organic matter, except the fat, by means of sulphuric acid. The fat is then separated by centrifuging and determined by reading off the percentage from the calibrations in the neck of the bottle used.

In the case of cow's milk or where sufficient human milk may be obtained, 17.6 c.c. of milk are measured into the bottle and 17.5 c.c. of sulphuric acid added. These fluids are then mixed by shaking and rotating the bottle in such a way that no curds pass into the neck of the bottle. As soon as the mixture becomes homogeneous and dark brown or even black in color, the bottles are placed in a special centrifugal machine and whirled for five minutes.

If the room be very cold it is advisable to fill the holders of the centrifuge with boiling water in order to keep the fat melted while it is being centrifuged. At the end of five minutes, centrifugation is discontinued and the neck of the bottle filled with boiling water. The melted fat will rise in the neck of the flask and may be read off from the calibration. In order to facilitate this the bottles are again centrifuged for one minute.

If a small amount of milk only is available the smaller tubes shown in cut may be

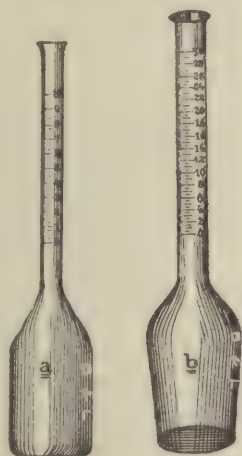


FIG. 163.—Babcock Bottles.
a, Milk bottle; b, cream bottle.

used. Milk is added to the mark five and sulphuric acid poured in so as to fill the body of the tube. It is usually necessary to add the milk and acid by means of thin narrow pipets, as the neck of the tube is too small to permit of easy entrance of the fluids otherwise. The milk and sul-

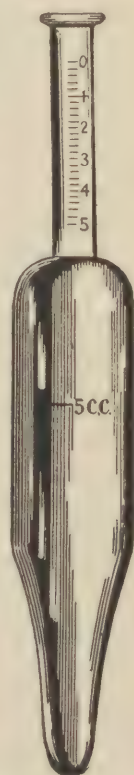


FIG. 164.—Bottle for human milk.

¹ See Brodrick-Pittard, *Biochem. Ztschr.*, 1914, LXVII, 382; Osborne and Wakeman, *Biol. Chem.*, 1915, XXI, 539; *Ibid.*, 1916, XXVIII, 1; Denis and Minot, *Ibid.*, 1918, XXXVI, 59; Pasch, *Zentralbl. f. Gynäk.*, 1921, XLV, 744. See Sheehy (*Biochem. Jour.*, 1922, XV, 703) for a study of the origin of milk fat and its relation to metabolism of phosphorus.

phuric acid are mixed by rotation of the tube until a homogeneous fluid results. The mixture is centrifuged for a few minutes, the neck of the tube being filled with a mixture consisting of equal parts of concentrated hydrochloric acid and amyl alcohol. The percentage of fat is then read off from the calibrations on the tube.

Extraction Method.

A few grams of dried washed sand are placed in the extraction shell of a Soxhlet apparatus and 10 c.c. of well-mixed milk are allowed to fall upon it drop by drop. This is dried at a temperature of 100°C. for one to two hours and is placed in the tube of the extraction apparatus. The fat is extracted in the usual way by the use of gasoline or anhydrous ether, complete extraction usually requiring from two to three hours. The apparatus is disconnected, the ether evaporated from the distilling flask, the residue in the flask dried at 100°C., and the flask and contents dried to constant weight in the desiccator. The difference between the original weight of the flask and its weight including the extracted residue, yields the amount of fat in the 10 c.c. of milk. This method is the most accurate one, but is not as convenient as the preceding for the general practitioner.

(9) Lactose.

In general routine analyses of milk the lactose may be determined by difference. By this is meant that subtraction of the values for the sum of water, ash, protein, and fat from 100 will yield the percentage of lactose. For clinical purposes this is usually sufficient, but for the more accurate work it does not give exact figures, as slight amounts of other undetermined substances are present.

For most direct determinations of lactose it is necessary that the larger portion of the protein material be removed previously. This may be done by the method outlined under Determination of Casein, Albumin, and Globulin; or for clinical purposes sufficiently accurate results may be reached by acidifying with acetic acid, boiling, and filtering. It is advisable always to take the time to saturate the mixture with carbon dioxid after the casein has been precipitated with acetic acid, as the results are more satisfactory.¹

The fluid is then titrated by the use of Bang's or Purdy's solution, using all the precautions mentioned under these tests in the section on Urine. The 35 c.c. of Purdy's solution are reduced by 0.02712 gram of lactose.

Folin and Denis² have applied the method of Folin and McEllroy to the determination of the lactose in milk. In a later communication Folin and Peck modified this latter method to a slight extent, as certain difficulties had been met with in the preparation of the salt mixture used in the test. This method is a very exact one and is especially recommended for the determination of lactose in milk as it is unnecessary to precipitate the protein

¹ See Jackson and Rothera, *Biochem. Jour.*, 1914, VIII, 1; Palmegiani, *Pediatrics*, 1914, XXII, 739; Hill, *Jour. Biol. Chem.*, 1915, XX, 175.

² *Jour. Biol. Chem.*, 1918, XXXIII, 521. See, also, Pacini and Russell, *Ibid.*, 1918, XXXIV, 505; Folin, Denis, and Minot, *Ibid.*, 1919, XXXVII, 349; Talbot, *Jour. A. M. A.*, 1919, LXXIII, 138; Denis and Talbot, *Am. Jour. Dis. Child.*, 1919, XVIII, 93.

before making the titration. It has been discussed in detail on page 328, but certain points, applying to milk, must be mentioned here. The milk must be diluted with water before making the determination. A dilution of 1:4 for cow's milk and 1:5 for mother's milk are satisfactory. The titration is made as follows: Into a large test-tube introduce 2.8 to 3.4 c.c. of the diluted milk (that is, nearly enough to produce complete reduction of the copper solution) 5 c.c. of the copper solution, a pebble (to prevent bumping), and 4 or 5 grams of the salt mixture previously mentioned. Shake well and boil gently for 4 minutes before adding any more milk. At the end of this time add more milk (0.02 to 0.1 c.c., depending on the amount of blue color remaining) and boil for 1 minute. The boiling should be for 1 minute after each addition of milk, the total boiling period being 5 to 7 minutes. The end reaction is determined by the decoloration of the solution and the precipitation of the white cuprous thiocyanate. In this test the amount of copper solution used is decolorized by 40.4 mg. of anhydrous lactose. Hence, 4.04 multiplied by the degree of dilution (4 or 5) and divided by the titration figure gives the per cent. of lactose present in the milk.

If the polarimeter is to be used for the estimation of lactose in milk the following procedure may be used. Fifty c.c. of the well-mixed milk are placed in a flask, 25 c.c. of a solution of neutral lead acetate are added, the flask is closed with a stopper through which passes a glass tube approximately 30 cm. in length. The mixture is then heated over a small flame to boiling. After the mixture has cooled it is filtered through a dry filter into a dry vessel and polarized.

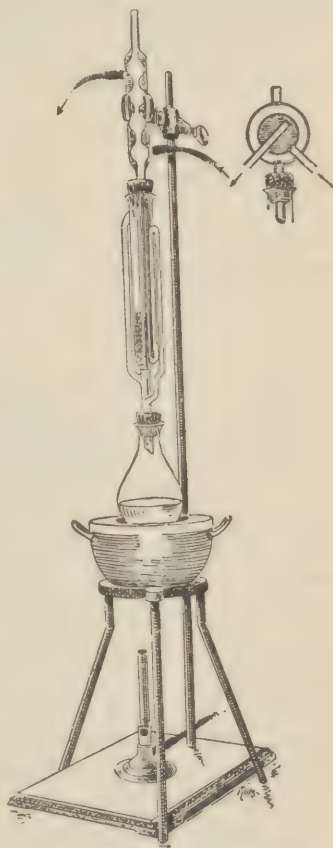


FIG. 165.—Soxhlet apparatus.
(Hawke.)

(10) Preservatives in Cow's Milk.

(a) Sodium Carbonate.

To hide the acid reaction of a spoiled sample of milk sodium carbonate is often added, and may be detected as follows: Ten c.c. of milk are mixed with ten c.c. of 96 per cent. alcohol and a drop of rosolic acid solution. Pure unadulterated milk produces a brownish-yellow color, but, in the presence of sodium carbonate or bicarbonate, a rose color is obtained. For greater precision in doubtful cases the questionable sample should be compared with known unadulterated milk. Phenol-phthalein solution may be used as an in-

indicator in place of rosolic acid. By this method 0.05 per cent. of carbonates may easily be detected.

(b) Salicylic Acid.

Twenty c.c. of milk are treated with two or three drops of sulphuric acid and shaken with an equal amount of ether. The greatest possible part of the ethereal solution is drawn off and evaporated, the residue extracted with 40 per cent. alcohol, filtered, and 5 c.c. of the filtrate treated with a few drops of ferric chlorid solution. A violet color shows the presence of salicylic acid or some other hydroxy derivative of benzol.

(c) Formaldehyd.

Two c.c. of concentrated sulphuric acid are placed in a test-tube and a drop of ferric chlorid solution added. A few c.c. of milk are allowed to run from a pipet upon the surface of the mixture in such a way that a distinct line of contact forms. A violet color at the point of contact of the two liquids is characteristic of formaldehyd in the presence of casein.

(d) Boric Acid and Borax.

Fifty c.c. of milk are alkalinized with milk of lime, evaporated to dryness and incinerated. The resulting white crystalline residue is treated with a few drops of tincture of turmeric and very dilute hydrochloric acid and is then dried on the water bath. The presence of the slightest trace of boric acid gives to the dry residue a beautiful vermilion or cherry red color. It is possible by this method to detect 0.001 per cent. of boric acid in milk. Only very dilute hydrochloric acid must be used in testing for boric acid, since the concentrated acid itself gives with tincture of turmeric a red color. The coloration produced by boric acid is distinguished from that produced by hydrochloric acid by the fact that it does not disappear by treatment with water in the cold, but only after long boiling, while the color caused by hydrochloric acid disappears as soon as it is diluted with water.

If the crystalline residue obtained as above described be treated with alcohol and the alcohol ignited, a flame tinged with a beautiful emerald-green color is obtained in the presence of boric acid.

III. BACTERIOLOGICAL EXAMINATION OF MILK

The bacteriological examination of human milk is frequently desirable from a clinical standpoint as milk may become contaminated as it passes along the lacteal ducts. In pathologic conditions many types of organisms, such as typhoid bacilli, pneumococci, and tubercle bacilli may be obtained, although the latter are extremely rare.

With cow's milk the chief question at issue is whether the milk contains sufficient bacteria to be harmful to the child. Aside from the presence of the tubercle bacillus in the milk of infected animals, large numbers of saprophytic organisms must find their way into this fluid and will, if in large numbers, influence the intestinal activity of the child. Moreover, it must be remem-

bered that many epidemics of milk-borne disease, such as typhoid fever and streptococcic sore-throat, may arise owing to improper handling of the milk or to inefficient pasteurization.¹

The methods of examining milk for bacteriologic differentiation are the same as for any other fluid and will be discussed in the next chapter. It is, not infrequently, a matter of importance to the general practitioner and of untold value to health officers to determine the number of bacteria in the milk supplied to the community. The outline of the method to be followed in this work is as follows:

Collect the specimen as freshly as possible. Keep it on ice from the time of collection until you are ready for the examination. Sterilize a quantity of distilled water in the autoclave. Plug a number of pipets (1 and 10 c.c.) with cotton and sterilize in the hot oven. A number of Erlenmeyer flasks, plugged with cotton, a 100 c.c. graduate, and a number of Petri dishes are sterilized at the same time.

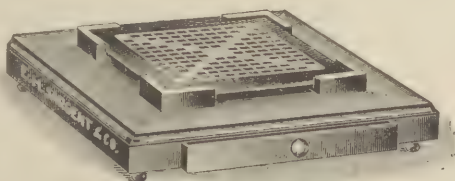


FIG. 166.—Wolfhügel's colony counter.

By means of the graduate place 99 c.c. of sterile distilled water in an Erlenmeyer flask, 90 c.c. in a second and 99 c.c. in a third and replace the cotton. By means of a sterile 1 c.c. pipet transfer 1 c.c. of the well-shaken specimen to flask number 1. The dilution of the milk in this first flask is, therefore, 1 to 100. Shake this dilution thoroughly and transfer 10 c.c. of this dilution, by means of a pipet, to the second flask containing 90 c.c. of water. The second dilution is 1 to 1,000. By means of a second 1 c.c. pipet transfer 1 c.c. of the 1 to 100 dilution to the last flask, containing 99 c.c. of water, thus obtaining a dilution of 1 to 10,000. These dilutions are usually all that are necessary: Others may be made as desired. In the examination of the best market milks, the 1 to 100 dilution is sufficient.²

Liquefy a number of tubes of litmus-lactose agar, litmus-lactose-bile agar and plain agar and cool them down to 40° to 45°C. Transfer 1 c.c. of each dilution of the milk to each of two sterile Petri dishes and add the liquefied culture medium. Mix by careful manipulation and allow the media to harden. Place in incubator for 48 hours and count the number of colonies, using a colony counter such as shown in the illustration. Multiply the number of

¹ See discussion of "Streptococcic Sore Throat" on page 45; also Rogers and Dahlberg, *Jour. Agric. Research*, 1914, I, 491. Shippen (*Jour. Am. Med. Assn.*, 1915, LXIV, 1289) shows, in confirmation of the work of De Jong and De Graef, that colon bacilli may survive the pasteurization process and are, therefore, not to be taken as evidence of later contamination.

² See Wells, *Am. Jour. Pub. Health*, 1919, IX, 956, for a discussion of standard method for bacteriological dilution.

colonies by the dilution to obtain the actual number of colonies per c.c. of original milk.¹

The number of bacteria in milk may vary from a few thousand to many millions. It is an impossibility to obtain a specimen which is sterile so that the presence of as high as 100,000 organisms should not be regarded as dangerous unless pathogenic types are present. If the freshly examined specimen contains between 10,000 and 50,000 bacteria per c.c., it is probable that all possible precautions have been taken to prevent contamination, although the writer has found some specimens showing a count of only 100 per c.c.

In the examination of milk it is customary also to make a cytological examination. It was formerly supposed that normal fresh milk contained only a few scattered leucocytes and epithelial cells, any marked increase in their number being taken as evidence of infection. However, by use of more careful methods it has been shown that the number of these cells may run as high as 500,000 or more and yet no abnormality be present. It is necessary in this work to distinguish between the ordinary leucocytes, which are constantly discharged in large numbers in the milk, and the true pus cells, which are evidence of disease. No detailed work along this line has been reported. The association of large numbers of bacteria, especially of staphylococci and streptococci, is probably evidence of infection of the animal and consequent unfitness of the milk for use, although the significance of the increase in cellular elements is not, by any means, settled. The methods used in this cytological examination are the same as outlined previously.²

¹ See Brew, New York Agricultural Exper. Station, Geneva, N. Y., Bull. 373, 1914; also, Goodrich, Jour. Infect. Dis., 1914, XIV, 512.

² See Lewis, Am. Jour. Dis. Child., 1913, VI, 225; Hachtel, Jour. Am. Med. Assn., 1913, XI, 565; Stokes and Stoner, Ibid., 1924; Breed, Bull. 380, New York Agricultural Exp. Station, Geneva, N. Y., 1914; Hewlett and Revis, Lancet, 1915, I, 855; Frost, Jour. Am. Med. Assn., 1915, LX, 821; Ibid., 1916, LXVI, 889; Levine and Emerson, Jour. Infect. Dis., 1916, XVIII, 143; Evans, Ibid., 437; Davis, Ibid., XIX, 236; Frost, Ibid., 273; Allen, Ibid., 712; Jordan, Jour. A. M. A., 1917, LXVIII, 1080; Simmons, Jour. Infect. Dis., 1919, XXIV, 322; Jones, Jour. Exp. Med., 1920, XXXI, 347; Ibid., 1921, XXXIII, 13; Barnes, Jour. Inf. Dis., 1921, XXVIII, 259.

CHAPTER XI

CLINICAL BACTERIOLOGY

I. GENERAL CONSIDERATIONS

In no field of medical or other scientific research have there been recent advances of such far-reaching importance to humanity as in the realm of Clinical Bacteriology. The study of the relationship of bacteria and their metabolic products to disease in general and the identification of the specific organism of many of our most dreaded diseases has led to such an enormous output of work upon the various ways in which the system may react to bacterial invasion, that our knowledge of the subject of immunity, both natural and acquired as well as active and passive, has advanced with leaps and

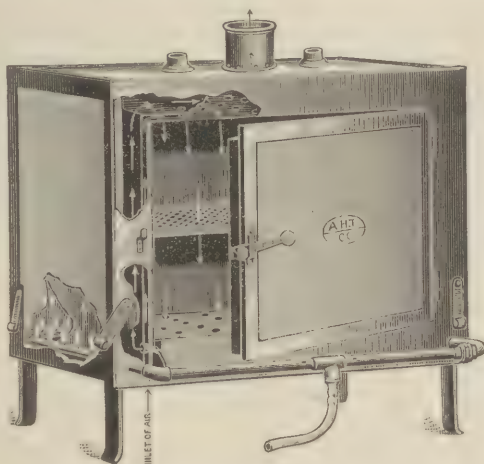


FIG. 167.—Lautenschlager Hot-air Sterilizer.

bounds. Indeed, so greatly has our knowledge increased that preventive medicine is fast taking the place of curative medicine. It is evident, therefore, that the scientific aspect of bacteriology, great as it is, is, for the general worker and for the public at large, overshadowed by the success attached to the practical application of the knowledge acquired from the hard and earnest work of scores of scientific investigators. Scientific and applied bacteriology must go hand in hand, each playing its important part in the struggle against disease.

The object of the writer in presenting this section is not to include a discussion of the advances in bacteriology, as this would, indeed, be absurd. He does wish, however, to present certain general features of the subject, so that

the student and practitioner may be given such a working knowledge as will enable them properly to perform and interpret many of the tests, which they may have occasion to undertake. Many such points have been discussed throughout this book but many have been omitted, so that the writer feels that this discussion will not be untimely.

II. STERILIZATION

As is well known, bacteria are practically ubiquitous, being found in air, water, food and soil. It is evident, therefore, that all material and glass-ware, which may be used in bacteriological work, must be clean and sterile before any reliable results may be obtained.

Glass-ware should be thoroughly cleaned by boiling in soapsuds or by soaking for a few hours in the following cleaning mixture.

Potassium bichromate,	60 grams.
Water,	300 c.c.
Concentrated sulphuric acid,	460 c.c.

After washing or soaking, rinse the glass thoroughly in running water and dry. Plug the test-tubes and flasks with non-absorbent cotton and place in a hot-air sterilizer or oven for one hour at a temperature of 170°C . The internal temperature of the sterilizer must be carefully regulated or the variation may be so great in different portions of the sterilizer that the more resistant organisms and spores will not be killed.

Culture media are best sterilized by the use of the autoclave, an apparatus for holding steam under pressure. The usual pressure used is 15 pounds, the media being kept in contact with steam at this pressure (temperature about 120°C .) for 15 to 20 minutes. The gas

is then turned off, and the apparatus allowed to cool.¹ While most media may be sterilized by this method, gelatin media would better not be heated longer than five minutes and blood-serum and carbohydrate-containing media may undergo chemical change if the heating is prolonged.

For this reason, such media as last mentioned or all media, in the absence of an autoclave, may be sterilized in the Arnold sterilizer, an apparatus so constructed as to produce steam from a small amount of water, the tank being fed by the water of condensation. As many bacterial spores are not killed by live steam, it is necessary to use the so-called discontinuous sterilization when

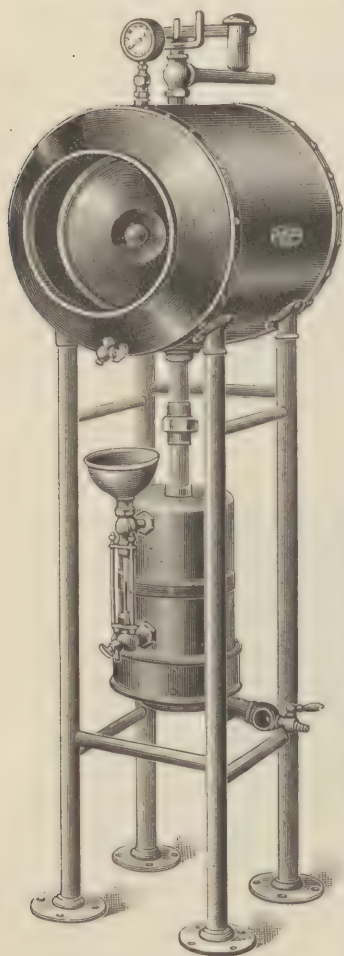


FIG. 168.—Autoclave.

¹ See Davis, *Am. Jour. Pub. Health*, 1920, X, 250.

the Arnold is employed. Heat the media for 30 minutes after the steam has filled the sterilizer and allow the media to stand at room temperature for 24 hours. Repeat the process on each of two other successive days. In this way spores develop into vegetative organisms, which are killed by the later heating.

Certain fluids, such as serum or ascitic fluid, cannot be sterilized by heating owing to coagulation. These are sterilized by passing through a Pasteur, Chamberland or Berkefeld filter.

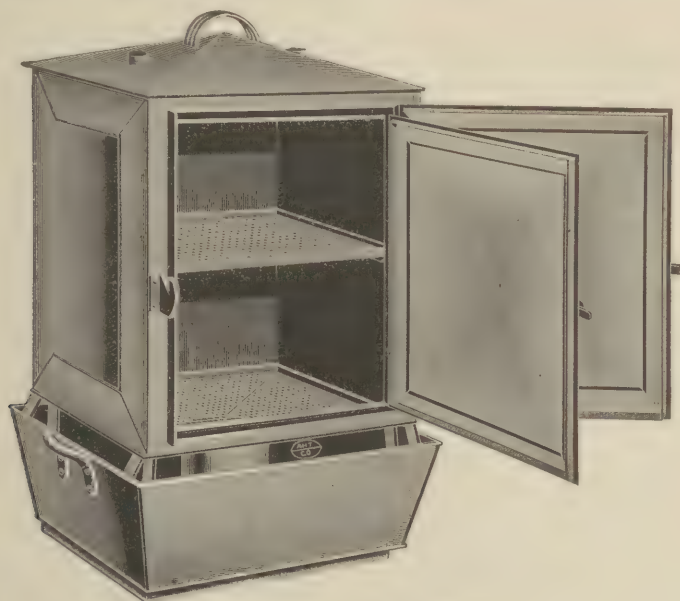


FIG. 169.—Arnold Sterilizer.

III. PREPARATION OF CULTURE MEDIA

For the development of bacteria certain factors are essential. The most important of these are (1) oxygen; (2) food; (3) moisture; (4) a proper reaction of the culture media; and (5) an optimum temperature for growth.

Regarding the question of proper food it is to be said that the number of culture media advocated is legion.¹ I cannot, therefore, attempt to give any but those in general use in laboratory routine. Many special media have been discussed in other sections of this book and will be referred to later in the discussion of special organisms.

Nutrient Broth (Bouillon; Beef Broth).

Infuse 500 grams of chopped lean meat with 1,000 c.c. of distilled water for 24 hours in the ice box. Instead of this meat, one may use 3 grams of Liebig's meat extract, but the medium is not as nutritious. Strain the infu-

¹ While it is true, as Rettger and his associates have shown, that purified unaltered proteins are resistant to bacterial attack, in the case of the simpler nitrogenous compounds we find that bacteria synthesize their own protoplasm when grown in synthetic media containing amino acids, or even ammonium phosphate or uric acid. See, Koser and Rettger, *Jour. Infect. Dis.*, 1919, XXIV, 301.

sion through cotton flannel, the juice being well pressed out. Add 1 per cent. peptone (10 grams). Warm on water bath, stirring until the peptone is dissolved. Heat over boiling water or steam for 30 minutes. Restore the original volume of fluid. Titrate the acidity of the broth, using N/20 sodium hydrate solution and phenolphthalein as an indicator.¹ Adjust the reaction

¹ The optimum reaction for such culture medium is one having a neutral reaction to litmus and a degree of acidity requiring 1 c.c. of normal (4 per cent.) sodium hydrate to neutralize 100 c.c. of the medium. This is known as +1. See Clark, Jour. Infect. Dis., 1915, XVII, 109.

Place 5 c.c. of the medium to be tested in a porcelain dish and add 45 c.c. of distilled water. Boil to expel carbon-dioxid. Add 1 c.c. of $\frac{1}{2}$ per cent. solution of phenolphthalein in 50 per cent. alcohol. Titrate, while hot, with N/20 sodium hydrate up to a faint but distinct pink color. Read from the buret the amount of N/20 NaOH used to neutralize the 5 c.c. of medium and calculate the amount of normal NaOH necessary to neutralize the entire quantity of bouillon.

Thus, if 1.5 c.c. of N/20 NaOH were used for 5 c.c., this would represent 30 c.c. for 100 or 300 for 1,000 c.c. But 300 c.c. of N/20 NaOH is equivalent to 15 c.c. of normal NaOH. As we desire the medium to have an end-reaction of +1 per hundred or 10 per liter, we must evidently add 5 c.c. of normal NaOH to bring this medium up to the proper standard.

While the above method is still used by some workers, it is more than probable that, ere long, this system will give way, entirely, as it has done in many laboratories, to the more reliable and scientific method of adjustment of culture media in terms of its P_H values. This method is discussed in great detail under Blood, but a few remarks, specially directed toward the application of this work to culture media are necessary. As Clark and Lubs have pointed out, the reference point in determining titratable acidities of culture media is, in reality, but a P_H point indicated by the tint of phenol phthalein. The particular tint adopted by different workers varies, so that the tint which is sometimes assumed is that at which phenol phthalein is least sensitive. Without a proper standard comparison solution of known P_H value the same worker can hardly avoid errors of 0.1 P_H in establishing his reference point for titratable acidity. The colorimetric method of adjusting the reaction of culture media on the basis of its P_H value is a return to the older method of adjusting to a given tint of an indicator but with more reliable indicators, a wider choice of indicators, a logical scale of reaction, and a clearer conception of the ends desired in the adjustment. The method of procedure is as follows: A measured 10 c.c. of the broth, whose reaction is to be adjusted, are placed in a clean test-tube which has been rinsed with a portion of the broth. To this is added $\frac{1}{2}$ c.c. of a 0.02 per cent. solution of phenol-sulphonephthalein (phenol red). Add, from a buret, a measured quantity of N/20 sodium hydrate solution, until the color of the mixture matches that of the standard solution, whose P_H value equals that chosen for the culture media. This comparison of color tints is made in a comparator, which consists of a simple wooden box with holes in the top for the test tubes and $\frac{1}{2}$ inch holes front and back to permit the passage of light through the solutions placed in the tubes. (While, for the usual adjustments of reaction of culture media, phenol red is satisfactory, yet cases may arise in which the range of P_H values indicated by this indicator does not fall within the scope of this indicator. In such cases other indicators, as mentioned under Blood must be used.) A simple calculation suffices to determine how much N/1 NaOH must be added to the remainder of the broth to bring the entire amount to the reaction of the chosen standard. Liquid media may be titrated either hot or cold. Gelatin media may be kept liquid at moderate temperature and adjusted like liquid media. The addition of agar to a medium should not appreciably affect the P_H of the medium, so that an agar medium may be adjusted before the addition of the agar. Blood serum or whole blood, added to an alkaline or acid medium, tend to approximate the final P_H to 7.3 or 7.5. Such an addition of serum tends to hold the H-ion concentration stable during growth of bacteria, owing to the presence of the "buffer" salts. As Clark and Lubs have pointed out, the adjustment of culture media by the colorimetric method should be checked by the electrometric methods, where absolutely accurate results are desired. However, for the usual run of bacteriological work, the colorimetric method suffices. The usual adjustment of culture media is to a P_H of 7.4. While the optimum and limiting value for the reaction of culture media in the case of the various organisms have not been worked out, Fennel and Fisher have shown the following: *Bacillus typhosus* and *B. paratyphosus* grow on agar as alkaline as P_H 9.6 and on one of an acidity of P_H 4. The optimum range for these organisms is 6.2-7.2. The *pneumococcus* seems to have a range of 7.2 to 8.2. *Streptococci* grow well at 7.6 to 7.8. *Meningococcus* has a rather narrow range of good growth, from 7.4 to 7.8. *Gonococcus* has an optimum of 7.6. The *diphtheria bacillus*, according to Davis, grows well in bouillon showing an hydrogen ion range of 6 to 8.1. See Clark and Lubs, Jour. Infect. Dis., 1915, XVII, 160; Jour. Biol.

to +1 by adding the calculated amount of normal sodium hydrate. Boil two minutes over free flame, stirring constantly. Restore loss by evaporation and filter through paper or absorbent cotton. Titrate and accurately adjust the final reaction to +1. Place the broth in test-tubes, using 10 c.c. in each tube. Plug tubes with cotton and sterilize in the autoclave.

Sugar-free Broth.

Make the infusion of meat (do not use meat extract) as above. Add to this 10 to 20 c.c. of a 24-hour broth culture of *Bacillus coli communis* and incubate at 37°C. for 18 hours. Boil the mixture to kill the organisms and proceed as above, using this material as if it were ordinary meat infusion.

Sugar Broths.

Add to the sugar-free broth above mentioned 1 per cent. of dextrose, lactose or other sugar. Adjust the reaction of these broths so that it is neutral to phenolphthalein. Sterilize by the discontinuous method.¹

Nutrient Gelatin.

To the nutrient broth add 10 per cent. of "gold label" sheet gelatin. Warm on water bath, stirring till gelatin is dissolved but not allowing the temperature to rise above 60°C. Heat over steam bath for 30 minutes and restore loss by evaporation. Titrate, after boiling one minute to expel carbon dioxide, and adjust the reaction to +1 as above described. Boil two minutes over free flame, stirring constantly. Make up loss by evaporation and filter through paper or absorbent cotton. If a clear filtrate be not obtained, it may be assured by cooling the mixture to 60°C. and stirring in the white of an egg beaten up in 30 c.c. of water. Titrate and adjust final reaction. Tube as above and sterilize in the autoclave at 120°C for five minutes. On being removed from the autoclave the gelatin is placed at once in the ice-box.

Nutrient Agar.²

Prepare the meat infusion as given under nutrient broth. Weigh the filtered infusion and add 2 per cent. of Witte's peptone. Warm on water bath stirring until the peptone is dissolved and not allowing the temperature to go above 60°C. While this mixture is being prepared, boil 15 grams of thread agar in 500 c.c. of water for one-half hour and make up the weight to 500 grams. Let it cool to 60°C. To 500 grams of the meat-peptone infusion add 500 grams of the 3 per cent. agar, keeping the temperature below 60°C. Heat over steam bath for 30 minutes and restore loss by evaporation.

Chem., 1916, XXV, 479; Jour. Bacteriol., 1917, II, 1, 109, and 191; Lubs and Clark, Jour. Wash. Acad. Sc., 1915, V, 609; 1916, VI, 481; Hurwitz, Meyer, and Ostenberg, Bull. Johns Hopk. Hosp., 1916, XXVII, 16; Kligler, Jour. Bacteriol., 1917, II, 351; Barnett and Chapman, Jour. A. M. A., 1918, LXX, 1062; Davis, Jour. Lab. and Clin. Med., 1918, III, 75; Kligler, Jour. Bacteriol., 1919, IV, 35; Avery and Cullen, Jour. Exper. Med., 1919, XXX, 359; Lord and Nye, Ibid., 389; Cohen and Clark, Jour. Bacteriol., 1919, IV, 407; McIntosh and Smart, Lancet, 1919, II, 723; Jones, Jour. Infect. Dis., 1919, XXV, 262; Fennel and Fischer, Ibid., 444; McIntosh and Smart, Lancet, 1919, II, 723; Brit. Jour. Exp. Path., 1920, I, 9; Grace and Highbarger, Jour. Inf. Dis., 1920, XXVI, 457; Medalia, Jour. Bact., 1920, V, 441; Foster and Randall, Ibid., 1921, VI, 143; Mellon, Acree, Avery and Slagle, Jour. Inf. Dis., 1921, XXIX, 1; Acree, Mellon, Avery and Slagle, Ibid., 7; Esty and Cathcart, Ibid., 29.

¹ See Holman, Jour. Infect. Dis., 1914, XV, 209; also Vedder, Ibid., 1915, XVI, 385.

² See Report of Committee on Standard Methods of Water Analysis, Jour. Infect. Dis., 1907, Supplement III, 108; also, Huber, Ill. Med. Jour., 1915, XXVIII, 207.

Titrate and proceed as given above for nutrient gelatin. Great difficulty is sometimes experienced in filtering this preparation, but if the filter paper and funnel be wet with boiling water, the medium usually passes through easily. Sterilize in the autoclave.

Glycerin Agar.

Prepare nutrient agar in the usual way. After filtering add 6 to 8 per cent. of glycerin. Mix thoroughly, readjust the titer if necessary and place in test-tubes. Sterilize in autoclave. This medium is preferable to plain agar in cultivating such organisms as the tubercle bacillus, which does not grow at all well on plain agar.

Lactose and Dextrose Litmus Agar.

To nutrient agar, prepared from sugar-free broth instead of nutrient broth, 1 per cent. of lactose or dextrose may be added just before sterilization, which must be in the Arnold sterilizer. Adjust the reaction to the neutral point to phenolphthalein. If the medium is to be used in tubes, the sterilized litmus solution or preferably azolitmin solution shall not be added until just before the final sterilization. If it is to be used in Petri dishes, the sterilized solutions shall not be added until the medium is ready to pour into the dishes. The litmus solution used is a 1 per cent. solution of Merck's purified litmus extract. Of this 5 or 6 per cent. is added. It is, probably, preferable to use instead of this, a 1 per cent. solution of Kahlbaum's azolitmin.

"Ameba" Agar.

This is the medium used by Musgrave and Clegg in cultivating the ameba and differs from nutrient agar in its strength. It is prepared in the same way but has the following formula:

Extract of beef,	0.3 to 0.5 gram.
Sodium chlorid,	0.3 to 0.5 gram.
Agar,	20.0 grams.
Water,	1,000.0 c.c.

The reaction is made -1, that is, it is 1 per cent. alkaline. Variations may be made in this medium, where the very delicate organisms are concerned, by adding a very small amount of peptone.

Blood Agar.

Prepare about 40 tubes of nutrient agar and, while still unsolidified, place them in a slanting position so that a large surface is given. Such tubes are called slants. Place 50 c.c. of nutrient agar in a flask and melt it. Allow it to cool to about 45°C. and add 15 c.c. of human blood, which has been drawn by venous puncture. Mix the contents of the flask by shaking and pour 2 c.c. of this mixture over the surface of each agar slant. Place these slants in position again so that the added material may harden. These tubes cannot be resterilized.

Instead of this method, which is preferable, one may add 1 to 2 c.c. of the human blood directly to 10 c.c. of agar melted and cooled to 40 or 45°C.

Mix by shaking and slant the tubes. This medium is especially valuable for routine work as it will grow practically all organisms.

Ascitic or Hydrocele Agar.

Dissolve 15 grams of agar in 1,000 c.c. of water. Filter and place 5 c.c. in each test-tube. Sterilize in the autoclave. To each agar tube add 5 c.c. of sterile ascitic¹ or hydrocele fluid and mix by rotating the tube. Cool so as to form slants. This medium cannot be resterilized. It is not always the simplest procedure to prepare this medium as the body fluid may be contaminated.

Litmus Milk.

Fresh milk, as pure as possible, is obtained and steamed for fifteen minutes in the Arnold. It is then placed in the ice box over night to allow the cream to separate. The cream is drawn off and the skimmed milk used for the medium. The reaction should be adjusted to +1. Add 1 per cent. azolitmin solution and tube 10 c.c. in test-tubes and sterilize for five minutes in the autoclave or in the Arnold for three successive days.

Blood-serum.

This is the medium devised by Löffler for the cultivation of the diphtheria organism and is very frequently used for others as well. It is prepared by adding 1 part of neutral nutrient broth containing 1 per cent. dextrose to 3 parts of sterile beef-serum. The mixture is then mixed thoroughly and placed in culture boxes or, preferably, in short tubes. In the latter it should be slanted. Coagulate at 75°C. After being held at this temperature until coagulated, the serum may be very slowly heated to about 95°C.

The above are the more important culture media used in routine work. Special media will be mentioned in discussing specific organisms.

IV. INCUBATION

Although bacteria may develop at temperatures above that of 37°C. (body temperature) or at much lower temperatures, yet the optimum temperature for most bacteria is 37°C. It is true that many organisms develop more luxuriantly and more rapidly at 20°C., but these bacteria are, for the most part, non-pathogenic.

It is essential, therefore, that some sort of apparatus be at hand, which will permit of the maintenance of a regular and uniform temperature of 37°C. Such an arrangement is called an incubator, many types of which may be obtained. Some of these are heated by gas and others by electricity, the latter being by far the more preferable. A heat-regulator is attached so that variations in the temperature inside the incubator may be reduced to a minimum. In these incubators must be placed a dish of water to supply the necessary moisture, the development of bacteria proceeding the better the more nearly saturated the atmosphere of the incubator.

Cultures when made upon the various media are placed in the incubator

¹ See Grace, Jour. Lab., and Clin. Med., 1920, V, 253.

for periods ranging from 24 hours to 10 days or more depending on the organism under investigation. As a rule 24 or 48 hours is the usual period of incubation. Owing to the rapidity of liquefaction of the medium, gelatin-containing media cannot be placed in a temperature of 37°C . Such a medium must be used at room temperature (about 20°C .).

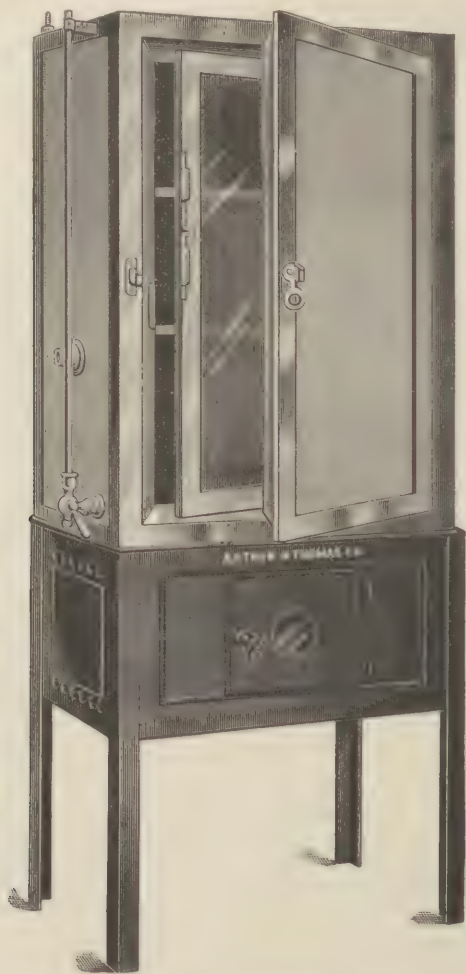


FIG. 170.—Incubator.

V. PREPARATION OF CULTURES

As previously stated, oxygen is absolutely essential for the development of all bacteria. Some organisms are capable of growth in free oxygen (atmospheric air), while others cannot live in such an atmosphere but obtain their oxygen from the culture media upon or in which they develop. The former bacteria are known as *aerobic* organisms, while the latter types are called *anaerobic* bacteria. The majority of the pathogenic organisms belong in the aerobic division, although many important pathogens are anaerobic.

A. Aerobic Organisms.

In this group we find the diphtheria bacillus, tubercle bacillus, influenza bacillus, typhoid bacillus, cholera spirillum, gonococcus, meningococcus, pneumococcus, and many others.

By means of the platinum needle, which is absolutely indispensable in bacteriological work, the material to be investigated is spread over the surface of the proper culture medium (in routine work agar or blood agar) and the cotton plug replaced. These plugs do not prevent access of air but keep out dust and contaminating bacteria. Place in the incubator and allow the culture to develop for 24 or 48 hours. Instead of the medium in the tubes, one may use liquefied agar or blood agar. Place the liquid material under investigation (such as milk or water) in a sterilized Petri dish and pour in the liquefied medium, which has cooled down to 40 or 45°C.

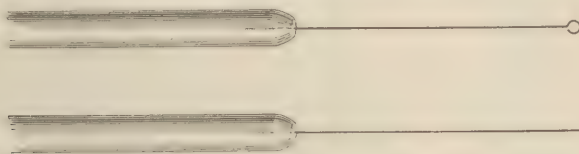


FIG. 171.—Platinum Needles.

Study the macroscopic characteristics of the culture and prepare smears from the culture for microscopic examination. If the cultures are pure, that is if only one type of organism is present, sub-cultures may be made upon special media in order absolutely to identify the organism. If several types are present, each kind of colony is transferred to other media and isolated in pure culture and then identified by sub-cultures. Where simple diagnosis is desired, these sub-cultures are rarely necessary, but where a pure culture is at issue, as in the preparation of a vaccine, very careful differentiation is essential.

B. Anaerobic Organisms.

In this class one finds the tetanus bacillus, spirillum pallidum, Vincent's spirillum, organism of anterior poliomyelitis and many others.

Cultures are made as in the case of aerobic organisms. However, these anaerobic types must develop in complete absence of free oxygen. To insure the absence of oxygen four methods are available.

(1) Boiling the culture media. Allow the medium to cool down to 40 to 45°C. and inoculate. This method is not at all reliable, owing to the possible access of air after boiling.

(2) Displacement of the oxygen of the air by an inert gas, usually by hydrogen. Inoculate the culture medium as above. Place in a larger vessel provided with entrance and exit tubes and tightly sealed. Pass a stream of purified hydrogen through the vessel continuously for several hours, close the stoppers and place the apparatus in the incubator.

(3) Absorption of the oxygen. This is the more commonly employed method. Place the culture tubes on a glass or metal support inside a larger tube or bottle. Place in the bottom of the bottle 10 grams of pyrogallol and

pour on it 100 c.c. of 1 per cent. sodium hydrate solution. Tightly stopper the bottle and place in the incubator.¹

(4) The use of a vacuum. This method consists in exhausting, with an air pump, the air in a large vessel containing the inoculated culture media. Close the tubes to prevent reentrance of air and place in the incubator. After the air is exhausted, hydrogen may be passed into the vessel, thus giving double assurance of freedom from oxygen.

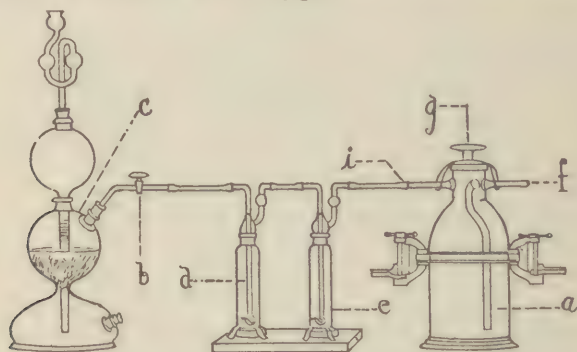


FIG. 172.—Anaerobic Cultivation in Hydrogen Gas (Heinemann). *a*, Novy jar; *b*, glass cock; *c*, gas generator; *d*, sulphuric acid; *e*, sodium hydrate solution; *f*, opening of Novy jar; *g*, stopper.

After the cultures have developed they are studied and examined as mentioned above. Sub-cultures and isolation of the different types are not so frequently essential with these anaerobic organisms, as the contamination is usually due to aerobic bacteria, which do not, of course, develop in these cultures.

VI. STAINING

The general methods of staining bacteria depend upon the special organism under investigation. As a rule Löffler's methylene blue (see p. 21) and Gram's stain (see p. 796) are the ones which give all the general information desired. Certain organisms, however, require special staining, which methods will be discussed under the specific organism.² Organisms are divided into two groups according to their reaction to Gram's stain, so that the writer would advise the constant use of this stain in order properly to classify an organism as positive or negative to this stain.

Staining of Spores.

Certain organisms show within their protoplasm a small bright refractile spot, known as a spore. These are very resistant and develop into vegetative organisms very rapidly. The spore is not stained readily, but when once stained is very resistant to decolorization, the body of the bacillus losing its

¹ The following method is very successful for routine work. Push the cotton plug into the tube a short distance, place dry pyrogallic acid on the upper portion of the cotton and pour on it 5 drops of 10 per cent. sodium hydrate solution. Stopper tightly with cork and seal it in with paraffin. See Barber, *Jour. Exp. Med.*, 1920, XXXII, 205; Thompson, *Jour. Inf. Dis.*, 1920, XXVII, 240; Itano and Neill, *Ibid.*, 1921, XXIX, 78; Kendall, Cook, and Ryan, *Ibid.*, 227; Hail, *Ibid.*, 317; Heller, *Jour. Bact.*, 1921, VI, 445; *Jour. Inf. Dis.*, 1922, XXX, 1, 18, and 33; Bushnell, *Jour. Bact.*, 1922, VII, 277.

² See Fleischer, *Jour. Med. Research*, 1917, XXXVI, 31. Conn. *Jour. Bact.*, 1921, VI, 253, recommends rose bengal as a general bacterial stain.

color before the spore does. In ordinary stained preparations the spore shows as an unstained spot in the body of the stained bacillus. Of course, the spore may be stained with the simple stain, if the action be prolonged or if the stain be heated, but this does not give clear differentiation.¹

Möller's Method.

Make a smear of the culture. Dry in air and fix in absolute alcohol for two minutes followed by chloroform for two minutes. Dry. Drop a few drops of 5 per cent. aqueous solution of chromic acid on the smear and leave for two minutes. Wash in water. Cover the smear with Ziehl's carbol fuchsin (p. 20) and warm for one minute. Wash in water and decolorize in 5 per cent. sulphuric acid for a few seconds, completing the decolorization, if necessary, with absolute alcohol. Wash in water. Stain with methylene blue for one-half minute. Wash in water, dry and examine. The spores are stained red and the body of the organism is blue.

Staining of Capsules.

Many methods have been advocated for this purpose, but the writer has found the method of Rosenow (see p. 693) the most satisfactory, giving beautiful pictures and uniform results.

Staining of Flagella.

Van Ermengem's Method.

Place the film for five minutes at 50°C. (or 30 minutes at room temperature) in the following freshly prepared solution.

20 per cent. aqueous solution of tannic acid,	60 c.c.
2 per cent. solution of osmic acid,	30 c.c.
Glacial acetic acid,	4 or 5 drops.

Wash in water and then in absolute alcohol. Cover the smear with $\frac{1}{2}$ per cent. aqueous solution of silver nitrate for a few seconds and, without washing, transfer the slide to the reducing bath for a few seconds. This reducing bath is as follows:

Gallic acid,	5 grams.
Tannic acid,	3 grams.
Fused sodium acetate,	10 grams.
Distilled water,	350 c.c.

Without washing, place the slide in the $\frac{1}{2}$ per cent. silver nitrate solution and move the liquid back and forth over the smear until this becomes black. Wash in water, dry and examine.

Successful preparations by this method, or any other, depend upon the obtaining of thin specimens.² Take a small amount of a young agar culture of the organism and make a homogeneous emulsion in a watch glass with distilled water. Place a drop or two of this emulsion on a perfectly clean glass slide and allow the drop to spread out. Dry in air and stain as above.

¹See Huntoon, Jour. Am. Med. Assn., 1914, LXII, 1397; Jour. Immunol., 1916, I, 126.

²See Lancereaux, Presse Méd., 1919, XXVII, 565; Shunk, Jour. Bact., 1920, V, 181.

VII. IDENTIFICATION OF ORGANISMS

After cultures have been prepared as above described and have been incubated for 24 to 48 hours, the diagnosis of a given infection may be possible by direct examination of the cultures and stained slides made therefrom.¹ This is true in cases in which search is being made for a specific organism without any reference to the associated bacteria. However, in the closer study of cultures, which is required in many cases, especially in the preparation of autogenous vaccines, sub-cultures on special media must be made in order properly to purify the culture and to permit of differentiation and recognition of the organism causing the infection. For this reason the writer deems it advisable to discuss briefly the cultural characteristics on special media and the means of absolute identification of some of the more important pathogenic bacteria.²

1. *Diphtheria Bacillus* (Klebs-Löffler Bacillus).

The medium best adapted for the development of the diphtheria bacillus is Löffler's blood serum.³ By means of a stout platinum needle or a sterile swab remove a portion of the suspicious material or membrane from the nose, throat or tonsils and spread this over the surface of the above culture medium. If the material is to be sent to a laboratory and no culture medium be at hand place the swab in a sterile test-tube or homeopathic vial and plug this with cotton. It is very essential that the proper material be obtained for these cultures or otherwise negative results will obtain. If necessary tear away the membrane in order to be certain of your material.

Place the culture in the incubator at 37°C. for six to eight hours and examine. The cultures should rarely be examined after more than 20 hours to insure the best results. The growth appears as a mass of grayish-white points or colonies varying in size from a small to large pin-head. They are

¹ It is important to remember that transmutations may appear within the groups of bacteria so that pneumococci, for instance, may change into true streptococci by variations in the culture medium, by changing the oxygen tension within the culture tube, etc. See Thiele and Embleton, *Ztschr. f. Immunitätsforsch. und exper. Therap.*, 1913, XIX, 643; Rosenow, *Jour. Infect. Dis.*, 1914, XIV, 1; Jordan, *Proc. Nat. Acad. Sc.*, 1915, I, 160. Further, it is to be held in mind that pleomorphism is not uncommon in cultures of bacteria, so that various types of organism may be found under conditions of altered environment, such as change in culture media, degree of acidity, etc., and that the new variety may retain the acquired peculiarities for some time. In this connection see Bergstrand, *Jour. Inf. Dis.*, 1920, XXVII, 1; Churchman and Kahn, *Jour. Exp. Med.*, 1921, XXXIII, 583; Florence, *Jour. Bact.*, 1921, VI, 371; Löhnis, "Studies upon the Life Cycles of the Bacteria" *Memoirs Nat. Acad. Sc.*, Sc. 16. 2nd. Mem., 1921. "Bacteriophage" or d'Herelle's phenomenon is a recently recognized phenomenon which is due to the activity of a substance occurring under certain conditions in bacterial cultures which has the property of destroying bacteria. Just what is the nature of this principle or its true function is still an unsettled point. See Twort, *Lancet*, 1915, I, 1241; D'Herelle, *Bull. Inst. Pasteur*, 1918, XVI, 97; C. R. Acad. Sc., 1917, CLV, 373. Rhodes (*Jour. Lab. & Clin. Med.*, 1922, VII, 288) gives a good review and bibliography of this subject.

² For a differential method of study of various bacteria by the addition of various anilin dyes to the culture medium see Churchman, *Jour. Exper. Med.*, 1912, XVI, 221; *Ibid.*, 1913, XVII, 373; Churchman and Michael, *Ibid.*, 1912, XVI, 822; Krumwiede and Pratt, *Ibid.*, 1914, XIX, 20 and 501; Simon and Wood, *Am. Jour. Med. Sc.*, 1914, CXLVII, 524.

³ See Drigalski and Bierast (*Deutsch. med. Wchnschr.*, 1913, XXXIX, 1237) who advise the addition of ox bile to this medium. Davis, *Jour. Lab. and Clin. Med.*, 1918, III, 75; Davis and Ferry, *Jour. Bacteriol.*, 1910, IV, 217; Bunker, *Ibid.*, 370; Klein, *Deutsch. med. Wchnschr.*, 1920, XLVI, 207; Frost, Charlton and Little, *Jour. Am. Med. Assoc.*, 1921, LXXVI, 30; Young and Crooks, *Am. Jour. Pub. Health*, 1921, CI, 241.

somewhat more opaque in their center than at their margin, which may be tinged a light yellow. The longer the period of growth, the larger the colonies, the more irregular their borders (early cultures usually show a regular margin) and the greater the tendency for the colonies to show radial striations. These colonies on blood serum always remain discrete and do not flow together.

Smears made from such cultures are stained with methylene blue and with Neisser's, Albert's or Gram's stain as given on page 42. Such smears show the organisms with the characteristic morphology and staining peculiarities given there. It is to be remembered that such cultures and smears may show no diphtheria bacilli, although the case is clinically one of diphtheria. This may be due to the taking of improper material for the culture, to the fact that antiseptic solutions have been used prior to the time of taking the culture or to the fact that too long a period of incubation has been used. Under the latter condition other organisms will so far outgrow the diphtheria bacilli that the latter are unrecognizable. For these reasons it must be insisted upon that a negative bacteriologic examination should never be regarded as excluding diphtheria.¹

In some very urgent cases recourse is made to the examination of direct smears from the swab without preliminary cultivation. This method rarely, if ever, gives results which may be relied upon but it occasionally does. It would seem to the writer that cultures should be insisted upon in all cases, as the time necessary for obtaining a growth is so slight that one is warranted in waiting for a more definite diagnosis. All suspicious throats, especially in children, should be regarded as diphtheritic until proven to be otherwise by several cultures. This precaution is best both for the patient and for his associates. See Schick's immunity test (p. 708).

It not infrequently happens that certain organisms, such as Hofmann's bacillus (pseudo-diphtheria bacillus) and the xerosis bacillus, complicate the diagnosis, as these organisms resemble the diphtheria bacillus in many ways. Cultures of Hofmann's bacillus, grown on Löffler's medium, show smaller and whiter colonies than those of the diphtheria bacillus. The growth of the former is much more rapid and there is not the same tendency for the colonies to take up the pigment of the medium and become yellowish in color. If Hofmann's bacillus be grown for 48 hours in glucose broth to which litmus has been added, no acid is produced; while the diphtheria bacillus readily produces acid (red coloration of medium). This fact has been mentioned on page 44, whereon are given the different sugars upon which these conflicting organisms act with production of acid but no gas formation. This fermentative test is of great value in differentiation.

Further, the morphology of Hofmann's bacillus is not the same as that of the Klebs-Löffler bacillus, although at times the two may become confusing. The former is shorter than the latter (although the latter occasionally shows a short form); the former is oval, stains deeply throughout except for one unstained portion and shows no granules. Occasionally one may observe a few

¹ See Bleyer, *Am. Jour. Dis. Child.*, 1920, XX, 445; Kolmer, Woody and Yagle, *Jour. Inf. Dis.*, 1920, XXVI, 179; Havens, *Ibid.*, 388.

beaded forms but these are rare. The more or less regular morphology of the pseudo-diphtheria bacillus is quite in contrast to the pleomorphic type of the diphtheria bacillus.

Negri and Mieremet¹ and Bunting and Yates² report the successful cultivation of a pleomorphic diphtheroid organism in cases of Hodgkin's disease, which organism had been previously demonstrated in the tissues by Fränkel and Much. The former workers used Bordet's potato medium and call the organism isolated the *Corynebacterium granulomatis maligni*, while the latter investigators employed Dorset's egg medium and glycerin-phosphate-agar and suggest the name of *Corynebacterium Hodgkini* (*Bacillus hodgkini*). Rosenow has reported the growth of these organisms on Löffler's blood serum and blood agar. The morphology of this organism is very pleomorphic: thus plump short rods, some so short as to resemble cocco-bacilli; small thin bacilli with polar staining; comma-shaped bacilli; granular rods of variable size; branching forms; club-shaped involution forms and large spherical types. The organism is Gram-positive, is non-acid fast and does not form spores.

2. Influenza Bacillus (Pfeiffer's Bacillus).

This organism is found most frequently in the sputum and nasal discharges of those affected with influenza. It is often recognizable in the routine sputum examinations, but it is often overlooked owing to its small size.

As hemoglobin is essential for the development of this organism, blood agar is the best medium upon which to cultivate it.³ In obtaining the speci-

¹ Centralbl. f. Bakteriöl., 1913, LXVIII, 292.

² Arch. Int. Med., 1913, XII, 236. See, also, Steele, Boston Med. and Surg. Jour., 1914, CLXX, 123; Billings and Rosenow, Jour. Am. Med. Assn., 1913, LXI, 2122; Kusunoki, Virchow's Arch., 1914, CCXV, 184; Yates, Bunting and Kristjanson, Jour. Am. Med. Assn., 1914, LXIII, 2225; Verploegh, Kehler and van Hoogenhuyze, Münch. med. Wchnschr., 1914, LXI, 1158; Mellon, Am. Jour. Med. Sc., 1915, CL, 245; Harris and Wade, Jour. Exper. Med., 1915, XXI, 403; Fox, Jour. Med. Research, 1915, XXXII, 300 and 325; Arch. Int. Med., 1915, XVI, 465; Rhea and Falconer, Ibid., 1915, XV, 438; Bloomfield, Ibid., 1915, XVI, 197; Bunting, Bull. Johns Hopkins Hosp., 1915, XXVI, 179; Olitsky, Jour. Am. Med. Assn., 1915, LXIV, 1134; Yates and Bunting, Ibid., 1915, 1053; Hatcher and Lemmon, Ibid., 1915, LXV, 1350; Torrey, Jour. Med. Res., 1916, XXXIV, 65; Bunting and Yates, Bull. Johns Hopk. Hosp., 1917, XXVIII, 151; Cunningham, Am. Jour. Med. Sc., 1917, CLIII, 406; Woolley, Jour. Lab. and Clin. Med., 1917, II, 523; Ebersson, Jour. Infect. Dis., 1918, XXIII, 1; Mellon, Jour. Med. Res., 1920, XLII, 61 and 111.

³ Brown and Orcutt (Jour. Exper. Med., 1918, XXVIII, 650) advise streaking the plates with hemolytic streptococci as this seems to facilitate growth of the influenza bacillus. Avery (Jour. A. M. A., 1918, LXXI, 2050) has shown that a 1:1000 sodium oleate added to the blood agar increased its value. This was prepared as follows: 5 c.c. of a 2% solution of neutral sodium oleate were added to 95 c.c. of a meat infusion agar. 1 c.c. of a suspension of rabbit red blood cells was then added while agar was hot and plates were then poured. The optimum pH for influenza bacilli is 7.2 to 7.5. See, also Pritchett and Stillman, Jour. Exper. Med., 1919, XXIX, 259; Winchell and Stillman, Ibid., XXX, 407. Although various workers, since the original work of Cantani, have believed that the influenza bacillus grows in media not containing blood or derivatives thereof, especially in symbiosis with other bacteria, yet this idea does not prevail among the majority of workers at the present time. Davis calls attention to the fact that "giant" colonies, the so-called "satellite" phenomenon, of these bacilli do occur about the central foreign colony of bacteria, yeasts, pieces of tissue, etc., yet by themselves they will not support growth of this organism. See Fildes, Brit. Jour. Exp. Path., 1920, I, 129; Putnam and Gay, Jour. Med. Res., 1920, XLII, 1; Stillman and Bourn, Jour. Exp. Med., 1920, XXXII, 665; Small and Dickson, Jour. Inf. Dis., 1920, XXVI, 230; Tunnicliff, Ibid., 405; Rosenow, Ibid., 409, 402, 504, 557, 507, 507 and 614; Davis, Ibid., 1921, XXIX, 171, 178 and 187; Williams and Povitzky, Jour. Med. Res., 1921, XLII, 405; Rivers and Poole, Bull. Johns Hopk. Hosp., 1921, XXXII, 202; Thjötta, Jour. Exp. Med., 1921, XXXIII, 763; Thjötta and Avery, Ibid., XXXIV, 455; Stillman, Ibid., 1922, XXXV, 7.

men for culture work, select a solid portion of the sputum and wash it several times with sterile distilled water to remove the excess of mucus. Or, if desired, make an emulsion of the sputum in sterile water. Take up the solid part of the washed sputum and rub it over the surface of a blood-agar plate in a Petri dish. Incubate at 37°C. for 24 or 48 hours. Macroscopic examination shows a number of small delicate dew-drop like colonies, which never become confluent. This organism is rarely found in pure cultures, the ancillary types being pneumococci, streptococci and staphylococci.¹ Other types of colonies will, therefore, be seen on the plates. Select a colony having the characteristics above mentioned and prepare smears as usual. The morphology and staining properties are as given on page 18.

3. *Pertussis Bacillus* (Bordet and Gengou's *Bacillus*).

The best medium for the cultivation of this organism is the potato medium of Bordet and Gengou, which is prepared as follows. To 200 c.c. of a 4 per cent. aqueous solution of glycerin add 100 grams of finely chopped potato. Heat in the autoclave for five to ten minutes. The supernatant fluid is now a concentrated glycerin extract of potato. To 50 c.c. of this extract add 150 c.c. of a 0.6 per cent. sodium chlorid solution and 5 grams of agar. Heat this mixture in the autoclave and, while hot, filter it into test-tubes, adding 2 to 3 c.c. to each tube. Sterilize in the autoclave. Allow the medium to cool down to 40 to 45°C. and add an equal amount of sterile defibrinated human or rabbit blood. Mix thoroughly and prepare slants of the mixture. This medium is especially valuable, also, in cultivating the influenza bacillus, gonococcus and meningococcus.

This bacillus should be looked for in the early stages of the disease when coughing begins. In the later stages it is not so easily found owing to the large number of other bacteria. Select a solid piece of the sputum and wash in sterile distilled water. Spread it over the surface of a Petri plate prepared from the above medium or have the patient cough directly on the culture medium spread in the Petri dish, the culture being thus obtained by droplet infection. These plates are more satisfactory for the bacilli of pertussis and influenza than are the slants, as these organisms require much oxygen. The primary cultures are difficult to obtain, as the growth is so scanty during the

¹ During the last few years quite an extensive epidemic of so-called "Spanish influenza" spread over the world, causing wide-spread destruction. The work on the epidemiology and etiology of this epidemic has been very extensive, the opinions being rather conclusive that the influenza bacillus was not entirely responsible for the trouble. I give only a few references, in this connection, as the mass of publications on the subject is too extensive to permit inclusion. See Mathers, Jour. A. M. A., 1916, LXVI, 30; Capps and Moody, Ibid., LXVII, 1349; Mathers, Ibid., 1917, LXVIII, 678; Bernstein and Loewe, Jour. Infect. Dis., 1919, XXIV, 78; Duval and Harris, Ibid., XXV, 384; Hirsch and McKinney, Ibid., 394; Gay and Harris, Ibid., 414; Wollstein, Jour. Exper. Med., 1919, XXX, 555; Lucke, Wight and Krine, Arch. Int. Med., 1919, XXIV, 154; Duval and Harris, Jour. Immunol., 1919, IV, 317; Parker, Ibid., 331; Valentine and Cooper, Ibid., 359; MacCallum, "The Pathology of the Pneumonia in the United States Army Camps During the Winter of 1917-1918," Monograph 10, Rockefeller Inst. Med. Res., 1919; Blake and Cecil, Jour. Am. Med. Assoc., 1920, LXXIV, 170; Gay, Jour. Lab. & Clin. Med., 1920, V, 543; Hall, Military Surg., 1920, XLVI, 564; Cecil and Blake, Jour. Exp. Med., 1920, XXXII, 719; Jordan and Sharp, Jour. Inf. Dis., 1920, XXVI, 463; Hall, Ibid., 1921, XXVIII, 127; Branham and Hall, Ibid., 143; Jordan and Sharp, Ibid., 357; Chesney, Ibid., XXIX, 132; Gordon, Ibid., 437 and 462; Anderson and Schultz, Jour. Exp. Med., 1921, XXXIII, 653.]

first 24 hours, and often in 48 hours, that little is visible. Sub-cultures made from this initial growth give a more copious development. On the third day the colonies appear white, slightly elevated and sharply outlined. From generation to generation the growth becomes quicker and more luxuriant. The morphology and staining characteristics of this organism have been given on page 29.

So close is the similarity of the influenza and the pertussis bacilli that it seems advisable to differentiate these organisms.¹ The growth of the former is quicker and less luxuriant than that of the latter. The colonies of influenza bacilli are slightly bluish and transparent, while those of the pertussis organism are whiter and thicker. If stroke cultures are made upon slanted potato-blood-agar, the growth of the pertussis bacillus is thicker than that of the influenza bacillus; its border is abrupt while that of the influenza bacillus is more spread out. The growth of the influenza bacillus is always transparent, shows an irregular border and has a moist appearance. Further, the pertussis bacillus hemolyzes the blood medium, so that the line of culture appears clearer than the surrounding. The influenza bacillus does not cause hemolysis.

Mallory, Horner and Henderson (Jour. Med. Research, 1913, XXVII, 391) have shown the etiologic relationship of this organism to pertussis, finding it in the characteristic lesions between the cilia of the epithelial cells lining the trachea and bronchi.

4. Typhoid Bacillus (Eberth-Gaffky Bacillus).

The characteristic cultural properties of this organism have been discussed on pages 139 and 689, while its agglutination reactions are given on page 710. The isolation and detection of the typhoid bacillus in the various excretions and secretions of the body is a matter of considerable difficulty, often calling upon all the resources at the command of the worker. Study of the cultural characteristics, of the agglutination reactions and the staining properties of this organism will readily differentiate it from the colon and associated bacteria, but careful work is necessary. It is questionable whether the typhoid bacillus has been isolated from water and milk supplies in a pure state, but this is not at all necessary as the detection of the colon bacillus, which is more easily identified, is all that is necessary to establish pollution. The typhoid bacillus may be found in the dejecta of convalescent and recovered patients for some time, so that careful measures should be adopted to prevent spread of disease through these carriers.²

5. Colon Bacillus (*Bacillus Coli Communis*).

This organism has been discussed on page 691 and its cultural characteristics and points of differentiation given. The colon bacillus is coming into great prominence, owing to its discovery in so many of the secretions and ex-

¹ See Morse, Boston Med. and Surg. Jour., 1916, CLXXV, 723; Chievitz and Meyer, Ann. de l'Inst. Pasteur, 1916, XXX, 503; Meyer, Ugesk. f. Laeger, 1916, LXXVIII, 1443; Ibid., 1921, LXXXIII, 523; Acta Paediat., 1921, I, 90.

² See Jordan, Jour. Am. Med. Assn., 1914, LXII, 1772; Hirschbruch, Berl. klin. Wchnschr., 1914, LI, 1176; Garbat, Jour. A. M. A., 1916, LXVII, 1493; Cumming, Ibid., 1917, LXVIII, 1163; Kendall and Hauer, Jour. Inf. Dis., 1922, XXX, 229.

cretions of the body under pathological conditions.¹ The use of vaccines made from cultures of colon bacilli is varied and the results usually excellent.

In the bacteriological examination of milk and water, the detection of the colon bacillus is of especial importance as an indicator of sewage contamination. In times past the custom has been to use, for this work, litmus-lactose agar for the plate cultures and glucose broth for the study of the fermentative properties of the organisms in the water. It is well known that both water and milk, as well as other fluids, contain acid- and gas-producing organisms which are not colon bacilli, so that one is not justified in calling all such bacteria colon bacilli. In order to eliminate these extraneous gas- and acid-producing bacteria, the use of bile has been advocated. The addition of bile to the usual media inhibits the other organisms but was not supposed to affect the colon bacilli. The media used in this later study and now adopted for all sanitary examinations of water and milk supplies are the lactose bile medium, consisting of 1 per cent. lactose dissolved in ox bile and sterilized, and lactose-bile-agar. The bile agar is prepared as follows. In 1,000 c.c. of fresh neutral ox bile dissolve 15 grams of agar, 10 grams of Witte's peptone and 10 grams of lactose, boiling as little as possible. When dissolved, filter the medium without titration, place in tubes and sterilize in the autoclave for three minutes. Rector² has advocated a slight modification of this medium, which he claims is much better. His formula is as follows:

Dried ox bile,	100 grams
Witte's peptone,	10 grams
Agar,	15 grams
Lactose,	10 grams
Neutral red (1 per cent. solution),	10 c.c.
Distilled water,	1000 c.c.

While it is unquestionably true that this bile medium has an advantage over non-bile media in that interfering organisms are eliminated and, therefore, the acid- and gas-producing organisms may more certainly be classified as colon bacilli, yet Jordan³ has shown that this medium exerts an inhibiting effect on colon bacilli themselves to the extent of $33\frac{1}{3}$ to 50 per cent. of the viable colon bacilli present in the material under investigation. Such being the case much more care must be exercised in reporting upon a specimen of water or milk, especially as to numbers of colon bacilli and as to the safety of such specimens for general use.

In recent years a vast amount of work has been done on the differentiation of the members of the colon group. From this work it appears that certain characteristics, such as the gas ratio, the Voges-Proskauer reaction and

¹ See Conradi and Bierast, *Kolle and Wassermann's Handbuch*, 1913, VI, 483; also Bornand, *Centralbl. f. Bakteriöl.*, Abt. 2, 1913, XXXVIII, 516.

² *Am. Jour. Pub. Health.*, 1913, III, 154. See, also, Bronfenbrenner, Schlesinger and Soletsky, *Jour. Bact.*, 1920, V, 79; Chen and Retger, *Ibid.*, 253; Ishii, *Ibid.*, 437; Novick, *Am. Jour. Pub. Health*, 1920, X, 305; Muer and Harris, *Ibid.*, 874; Levine, *Ibid.*, 1921, XI, 21; Wagner and Monfort, *Ibid.*, 203; Koser and Skinner, *Jour. Bact.*, 1922, VII, 111; Kendall and Bly, *Jour. Inf. Dis.*, 1922, XXX, 239.

³ *Jour. Infect. Dis.*, 1913, XII, 326.

the Methyl Red Test of Clark and Lubs are especially valuable in this differentiation. Rogers, Clark and Davis and Rogers, Clark and Evans, using a slight modification of the accurate method of Keyes for the collection and examination of the gases produced by members of this group in carbohydrate media, show that bacteria of the colon-aerogenes family may be sharply divided into two groups on the basis of the gas ratio. One group produces CO_2 and H in the constant ratio $\text{CO}_2:\text{H}_2 = 1.06$, the members of this group being termed "the low ratio organisms." The second group produces much more CO_2 than H and furnishes a ratio which varies from about 1.90 to 3.00, such organisms being called "the high ratio organisms." In this study there were also found some organisms, which produce CO_2 but no hydrogen. These organisms may be distinguished from the colon-aerogenes family by cultural characteristics. As they liberate no hydrogen, the gas ratio is infinity, the organisms being termed "the infinity ratio group."

The Voges-Proskauer reaction is a colorimetric test, observed after addition of 10 per cent. sodium hydrate to the fluid in the fermentation tube for the purpose of absorbing the CO_2 and thus, determining the gas ratio. If the alkalized carbohydrate medium, in which organisms of this group have been grown, be allowed to stand for 24 hours at room temperature a reddish color resembling eosin will appear, when this Voges-Proskauer reaction is positive. While it is customary to allow the tube to stand for 24, and by some for 48 hours, yet this would seem to be unnecessary as Levine, Weldin, and Johnson have shown that a faint reaction may be recognized within 1 hour and, after 5 hours, as distinct a reaction as at the expiration of 24 hours. The true colon bacilli give negative reactions with this test, while the bacillus cloacæ and associated members of this group show a positive result. That is a positive Voges-Proskauer reaction is given by those members of the "high gas ratio group," showing large production of CO_2 and small amounts of H. This reaction appears to be due to a special decomposition of glucose with the formation of acetyl-methyl-carbinol ($\text{CH}_3\text{-CHOH-CO-CH}_3$). This product is further oxidized, in presence of air, to diacetyl ($(\text{CH}_3\text{CO})_2$), which in the presence of NaOH reacts with the peptone of the culture medium to form the eosin-like compound. Levine, Weldin, and Johnson have introduced a fuchsin-aldehyd reaction, which is, also, used in the correlation of members of this group. The test is performed as follows: To 2 c.c. of a 72 hour culture of the organism in the 0.5 per cent. carbohydrate-peptone-dipotassium phosphate medium of Clark and Lubs were added 2 or 3 drops of basic fuchsin decolorized with sodium sulphite. A slight pink coloration is recorded as negative, while a distinct red or cherry color is considered positive. As compared with the results of the Voges-Proskauer reaction, this latter test shows that among 124 cultures, which gave the positive Voges-Proskauer, only 10 were positive with the fuchsin test; while 44 of 45 cultures of Voges-Proskauer negative strains showed positive results with the fuchsin test.

Clark and Lubs have introduced a further colorimetric test, which serves to differentiate members of the colon-aerogenes family. The medium used in this test consists of 0.5 per cent. Witte peptone, 0.5 per cent. pure dextrose,

and 0.5 per cent. K_2HPO_4 . While the authors state that Witte peptone must be used, other workers have found other peptones quite as effective, especially the peptone of Parke, Davis & Co. and the Difco product. The test is performed as follows: Pour about 5 c.c. of the culture in the 10 c.c. of the above culture medium (which has been incubated for at least 3 days) into a clear glass test tube and add 2 to 4 drops of paranitrophenol solution (0.2 gram dissolved in 30 c.c. alcohol and diluted to 500 c.c. with distilled water) and add 1 to 2 drops of methyl-red solution (0.1 gram dissolved in 300 c.c. alcohol and diluted to 500 c.c. with distilled water) to the remaining $\frac{1}{2}$ of the culture medium. Record the color changes. In a study of 190 cultures by this method, the following results were noted: Without exception, the "low ratio organisms" (true colon bacilli) were perfectly colorless to paranitrophenol and brilliantly red to methyl red; without exception the "high ratio organisms" were distinctly colored with paranitrophenol, while all but five were distinctly yellow to methyl red. In other words the "low ratio cultures" are distinctly acid, while the "high ratio cultures" are distinctly alkaline. It is to be said that the methyl red gives sharper results, so that this is recommended over the paranitrophenol. In comparing this test with the Voges-Proskauer, we find that positive methyl red organisms show negative Voges-Proskauer reactions and vice versa. The test is really a method of distinguishing the H ion concentration of the culture media in which members of this group are grown.¹

6. Dysentery Bacillus (Shiga-Kruse Bacillus).

In bacillary dysentery certain organisms are found which closely resemble the typhoid and colon bacilli in morphology but which differ in certain cultural characteristics from the latter bacteria. In the course of an epidemic of dysentery in Japan, Shiga discovered this bacillus, but it remained for Kruse to elaborate and fix its characteristics. Since that time, similar organisms have been found in various epidemics throughout the world, but in many cases certain differences were observed in the behavior of the organisms toward different sugar-containing media. On the basis of fermentative tests with acid production in sugar-broths, and, also, upon variability in the agglutination reactions of the organisms isolated, at least four distinct types of the dysentery bacillus must be considered as established.

I. Shiga-Kruse type.

II. Flexner-Strong type.

III. Hiss-Russell, El Tor I strain, "Y" type.

IV. Harris-Wollstein type.

¹ In this connection see Voges and Proskauer, *Ztschr. f. Hyg.*, 1898, XXVIII, 20; Harris, *Bull. de l'Inst. Pasteur*, 1906, IV, 250; Harden, *Proc. Royal Soc.*, 1906, LXXXVII, 424; Keyes, *Jour. Med. Res.*, 1909, XXI, 69; Frieber, *Centralbl. f. Bakteriöl.*, Abt. I, Orig., 1913, LXIX, 437; Kligler, *Jour. Infect. Dis.*, 1914, XV, 187; Browne, *Ibid.*, 580; Rogers, Clark and Davis, *Ibid.*, 1914, XIV, 411; Rogers, Clark, and Evans, 1914, XV, 99; *Ibid.*, 1915, XVII, 137; Clark and Lubs, *Ibid.*, 160; Levine, *Ibid.*, 1916, XIX, 773; Levine, Weldin, and Johnson, *Ibid.*, 1917, XXI, 39; Burton and Rettger, *Ibid.*, 162; Nelson, *Jour. Am. Chem. Soc.*, 1917, XXXIX, 515; Bronfenbrenner and Davis, *Jour. Med. Res.*, 1918, XXXIX, 33; Koser, *Jour. Infect. Dis.*, 1918, XXIII, 377; Salter, *Ibid.*, 1919, XXIV, 260; Winslow, Kligler, and Rothberg, *Jour. Bacteriol.*, 1919, IV, 429.

The following table, taken from Jordan,¹ will show at a glance the variations in the action of these similar organisms.

Bacillus dysenteriae	Acid production from:				Indol production
	Mannite	Maltose	Saccharose	Dextrose	
Type I (Shiga-Kruse).....	—	—	—	+	—
Type II (Flexner-Strong).....	+	—	+	+	+
Type III (Hiss-Russell).....	+	—	—	+	+
Type IV (Harris-Wollstein).....	+	+	+	+	+

The detection of these organisms in the stool is not a matter of great difficulty, by cultural methods, if the case is examined relatively early in its progress. However, after some time the examination becomes more and more uncertain until the organisms are rarely found after the third week. The material for the preparation of cultures is obtained from a freshly passed stool. Flecks of mucoid material are selected and placed in sterile water or physiologic salt solution, in order to remove the colon bacilli with which the material is contaminated. This washing is repeated three or four times, fresh water being, of course, added each time. If there be no visible mucus in the stool, emulsify a portion of the stool in nutrient broth. The washed mucoid particles, or a portion of the fecal emulsion, are now rubbed over the surface of three or four litmus-lactose agar plates or of plates made of Drigalski and Conradi's medium (see p. 139) without the addition of the crystal violet. Allow these plates to dry in the air by keeping the cover slightly open for one-half hour, place them in the incubator at 37°C. with the bottom side up and examine at the end of 24 hours. Any colon bacilli left after the washing, and there are always some, will appear as red colonies with a gradual shading of color into the blue medium. The Shiga bacillus does not change the color of the medium, its colonies appearing as translucent, iridescent, dew-drop like growths with irregular margins and centers more opaque than the edges. This growth is very similar to that of the typhoid bacillus on this medium. Test one of the latter colonies for motility of the organisms by the hanging drop method and, also, test its agglutination with a potent dysentery serum. If these tests are satisfactory, namely, if no motility is observed and agglutination is definite, sub-cultures of these colonies are made upon slant agar, neutral red-glucose-agar (glucose agar to which three or four drops of a saturated aqueous solution of neutral red are added), litmus-milk and plain milk. The cultures on agar and neutral red-glucose-agar

¹ General Bacteriology, Philadelphia, 1913. See, also, Lentz, Kolle and Wassermann's Handb., 1913, III, 927; Barber, Philippine Jour. Sc., 1913, VIII (B), 539; Blühdorn, Deutsch. med. Wchnschr., 1914, XL, 782; Monatsschr. f. Kinderhke., 1914, XIII, Orig., 37; Schild, Ibid., 51; Musgrave and Sison, Philippine Jour. Sc., 1914, IX (B), 241; Flexner and Amoss, Jour. Exper. Med., 1915, XXI, 515; Sutherland, Brit. Med. Jour., 1916, II, 142; Dünner and Lauber, Berl. klin. Wchnschr., 1916, LIII, 1266; Foucar, Mil. Surg., 1916, XXXVIII, 534; Smillie, Am. Jour. Dis. Child., 1917, XIII, 337; Gibson, Jour. Royal Army Med. Corps, 1917, XXVIII, 615; Benians, Jour. Path. & Bact., 1920, XXII, 171; Twort, Brit. Jour. Exp. Path., 1920, I, 237; Davison, Jour. Exp. Med., 1920, XXXII, 651; Levine, Jour. Inf. Dis., 1920, XXVII, 31; Kendall and Hauer, Ibid., 1922, XXX, 226.

show the same characteristics as do those of the typhoid bacillus, but the lack of motility excludes this latter organism. These cultures have a peculiar sperm-like odor. On litmus-mannite-agar the Shiga bacillus produces no acid, while the typhoid bacillus develops a distinct red color. Litmus-milk is not changed in color, nor is plain milk coagulated.

It will be seen, therefore, that the Shiga bacillus differs from the typhoid bacillus in its action toward litmus-mannite-agar and, especially, in its lack of motility and its different agglutination reactions. From the colon bacillus, the cultural characteristics differentiate it sharply.

The morphology and staining properties of this organism have been given on page 141. It is Gram-negative, does not form spores, nor does it possess flagella. In smear preparations, the dysentery bacillus resembles the typhoid bacillus, but the former is thick and often polymorphous.

Culturally the other types of dysentery bacilli are differentiated from the Shiga bacillus by the fact that their cultures on litmus-lactose-agar are more irregular and have a reddish-violet tinge, while the latter organism shows colonies of a translucent dew-drop like appearance. Further, the Shiga bacillus does not produce indol, while all the other types, as well as the colon bacillus, do. In confirming this differential diagnosis, the agglutination tests are valuable. The Shiga bacillus should agglutinate in a dilution of 1 to 20 of the patient's serum, while the Flexner type agglutinates at a dilution of 1 to 80. For differentiation of the other types, the fermentative tests must be utilized, as agglutination is not typical.

7. Pathogenic Anaerobes.

"In spite of the accumulation of much knowledge on the subject of anerobes, a study of bacteriological literature before the war shows clearly that the descriptions published by different writers were for the most part widely divergent, and agreed in only a few instances. From the frequency with which *Bacillus welchii* (*B. perfringens*) was found, apparently in pure culture, it was considered that this was the chief etiological agent, and it was actually named 'the bacillus of gas gangrene.'" As stated in the Report of the Committee upon Anaerobic Bacteria and Infections, No. 39, of The Medical Research Committee, a careful scrutiny of the discharges from wounds by more refined methods during 1916 and 1917 revealed the fact, however, that gas gangrene could be caused by a series of pathogenic anaerobes acting singly or in combination with each other or in association with certain well defined non-pathogenic types. The work of the last few years has lead to the recognition of many new types and to the more exact characterization of the older ones of this group of organisms. By newer methods of study it is now possible to obtain surface cultures of these anerobes and to study them with much greater care and accuracy.

The pathogenic and many of the non-pathogenic anerobes, which may infect wounds contaminated with soil, have been isolated and described. The list of these is a long one, but the majority seem to be non-pathogenic. The principal pathogenic types are *Bacillus welchii*, *vibrio septique*, and

Bacillus œdematiens in this group of wound infecting organism, while the *Bacillus tetani* must come in for separate discussion. These 3 first types, either acting alone or in combination, seem to be responsible for most of the acute cases of gas gangrene. They may be helped in their pathogenic activity by the non-pathogenic anaerobes, for example by the *Bacillus sporogenes*. While our knowledge of this enormous group of organisms is still small, yet we are gradually accumulating information, which will enable us to cope with emergency conditions in the future with much more assurance. Especially important in the elucidation of some of the obscure points in this study have been the researches of Weinberg and Séguin, McKee, Stokes, McIntosh, Miss Robertson, Bull and Stoddard, with many others. In recent times Miss Heller has made several extensive studies in this field, which promise to bring more order out of the chaos now existing.¹

a. *Bacillus Aërogenes Capsulatus*.

This organism, which is also known as the *Bacillus Welchii* and the "*Gas Bacillus*," has assumed considerable importance during the recent war owing to the number of cases of infection arising from contamination of wounds with it. Hitherto it has been known as a contaminating organism of soils and drinking water and has been especially associated with cases of emphysematous gangrene. During the war, the infection with this organism reached such a degree owing to the fact that the clothing of the men was contaminated with the organism or its spores due to the trench life and, also, to the fact that the soil had been so intensely cultivated prior to the war. An interesting report on the contamination of the clothing has been made by Simonds² who states "Spores of the *Bacillus welchii* group of bacteria were found on 100 per cent. of the uniforms of Belgian soldiers who had come directly from the trenches, and in the meshes of all the samples examined of the new cloth from which the uniforms were made." In a study of infection with this organism Taylor³ suggests that the fatal cases of infection pass through the following phases: Phase 1, dormant stage; localized infection of the wound; Phase 2, stage of acute gaseous distention, the result of obstruction to the escape of the gas generated locally in the wound; Phase 3, explosive stage; this is characterized by the rapid extension of the swelling associated with subcutaneous crepitation: Phase 4, stage of systemic intoxication; Phase 5, stage of septicemia. The terminal invasion of the blood by the bacilli, when it happens, is probably a very late phenomenon, occurring approximately at the time of death. The first stage represents the common type seen in fresh wounds. The great majority of cases do not pass beyond this stage. When, however, for any

¹ A full bibliography of this subject will be found in McIntosh, "Classification of Anaerobic Bacteria of Wounds," Spec. Rep. No. 12, Med. Res. Comm., 1917 and in Report of Committee upon Anaerobic Bacteria and Infections, Spec. Res. No. 30, Med. Res. Comm., 1919. For later work along these lines see Robertson, Jour. Path. & Bact., 1920, XXIII, 153; Wolf, *Ibid.*, 254; Henry and Lacey, *Ibid.*, 281; Heller, Jour. Inf. Dis., 1920, XXVII, 385; Jour. Bact., 1921, VI, 445 and 521; Hall, Jour. Inf. Dis., 1921, XXIX, 321; Heller, Jour. Bact., 1922, VII, 1; Barney and Heller, Arch. Surg., 1922, IV, 470; Heller, Jour. Inf. Dis., 1922, XXX, 1, 18 and 33.

² Jour. Exper. Med., 1917, XXV, 819.

³ Jour. Path. and Bacteriol., 1917, XX, 384.

reason, the free escape of the gas becomes interfered with, the second phase, with its clinical picture of gas gangrene develops rapidly and passes frequently within a few hours into the third stage. If sufficient drainage be established at this point for the escape of gas, and too large a mass of muscle has not been destroyed, recovery is usual. It is at this time or before that the value of X-ray examination is so great from the diagnostic point of view. It is true that, while the opportunities for infection with this organism were so great, there was a relatively small incidence of gas gangrene in infected wounds. The reasons for this were, probably, the rapid surgical interference which was given and the drainage of the wound. Bulloch and Cramer¹ would have us believe that certain influences, particularly chemical, produced local changes leading to local breakdown of the normal defensive mechanism against the bacteria of this group. For this phenomenon, they have coined the word "cataphylaxis" or "defense rupture."

The gas bacillus is widely distributed, being commonly found in the intestinal tract of the higher animals, and in soil, dust, sewage, river-water² and milk. While the cause of emphysematous gangrene, it has been observed in certain uterine infections, and infections of the gastro-intestinal, genito-urinary, and biliary tracts (Jordan). The development of gas in the internal organs, especially the liver, the so-called "foamy organs," observed post-mortem is often due to this organism. As Jordan³ states, "great confusion has reigned in the field of identification and nomenclature. The following names probably refer to the same organism: *B. welchii*, *B. aerogenes capsulatus*, *B. phlegmonis emphysematosæ*, *B. enteritidis sporogenes*, and *B. perfringens*. A bacillus found by Achalme and others in cases of acute rheumatism, and regarded by them as standing in causal relation to that affection, is almost certainly identical with *B. welchii*."

This organism was originally described by Welch and Nuttall⁴ and later by Fraenkel. It is a plump, rather long bacillus (3 to 6 μ), occurring in chains and singly; it is non-motile, anaerobic, and stains by Gram's method. Capsules are usually present in preparations made from the organs or body fluids. In old cultures gram-negative types and many pleomorphic involution forms may be found. Spores are formed by some races and are particularly prone to appear on blood-serum. Gas is produced in dextrose, lactose, and saccharose media, but not in mannite, a small amount of gas may be formed, also, from protein substances. Hydrogen predominates in the gas produced from sugar media, the ratio of H and CO₂ ranging from 2:1 to 3:1. Milk is coagulated with abundant gas production and strongly acid reaction (stormy fermenta-

¹ Proc. Royal Soc. London, 1919, XC, 513.

² Lerner (Jour. Am. Med. Assoc., 1922, LXXVIII, 276) reports its presence in a public water supply.

³ General Bacteriology, Saunders, Phila.

⁴ Bull. Johns Hopk. Hosp., 1892, III, 81; Fraenkel, Centralbl. f. Bakt., 1893, XIII, 13 see, also, Welch and Flexner, Jour. Exper. Med., 1896, I, 5; Dunham, Bull. Johns Hopk. Hosp., 1897, VIII, 68; Welch, Ibid., 1900, XI, 185; Kamen, Centr. f. Bakt., 1904, XXXV, 554 and 686; Arch. f. Hyg., 1905, LIII, 128; Herter, Jour. Biol. Chem., 1906, II, 1; McCampbell, Jour. Infect. Dis., 1909, VI, 537; MacNeal, Latzer, nad Kerr, Ibid., 571; Blake and Lahey, Jour. A. M. A., 1910, LIV, 1671; Jablona, Jour. Lab. & Clin. Med., 1920, V, 374; Kendall, Day and Walker, Jour. Inf. Dis., 1922, XXX, 141.

tion); the casein is not digested. The acids produced are butyric and allied acids, but lactic acid is scanty. For this reason, there is a typical odor of butyric acid in milk and glucose-agar cultures. Most varieties liberate hemoglobin when grown in bouillon to which blood has been added. Smith has advocated the simple method of detecting this organism by adding a bit of sterile normal liver or other animal tissue to broth in the fermentation tube. Gas usually develops abundantly in 24 hours at 37°C., if the inoculating material contains the spores of *B. welchii*. This organism grows well on all ordinary culture media, rather rapidly at 37°C., but is strictly anaerobic. The colonies are greyish-white on agar, and gelatin is liquefied. In glucose-bouillon the growth is first evidenced by a diffuse cloudiness with the fluid later becoming clear and a whitish viscid sediment settling out. While there are some similarities between this organism, especially as observed in water and milk analyses, and the bacillus coli communis, yet there should be little difficulty in differentiating them by recourse to the above cultural methods and a search for the spores of the *B. welchii*.

While this organism is pathogenic for man, under certain conditions, yet it does not affect the lower animals universally. Rabbits and mice are practically immune to simple inoculation. Dead rabbits are, however, of special value in a study of this organism. If a suspected material be injected intravenously into a rabbit and the rabbit be killed a few minutes thereafter and the animal incubated at 37°C., gas is produced in a few hours throughout the body, the liver showing gas within 4 to 6 hours after inoculation. Guinea pigs show a variable reaction; if injected subcutaneously they sometimes die with subcutaneous emphysema accompanied by extensive necrosis and tissue digestion; sometimes they develop local abscesses; and sometimes are unaffected. Pigeons are, also, highly susceptible.

b. Bacillus Œdematis Maligni.

This organism is probably identical with *Vibrio septique* isolated in 1877 by Pasteur, while the name of Bacillus of malignant edema was given to it by Koch in 1881. In most of the writings during and since the war, the name *Vibrio septique* seems to take precedence. Moreover, this organism has been confused with the *Bacillus anthracis symptomatici*, or, as it is sometimes called, *Bacillus chauvoei*, but there can be little question that these organisms are not identical. Recently Miss Robertson has shown that there are probably 3 types of the *Vibrio septique*, but that none of these can be regarded as identical with the *Bacillus chauvoei*. Fermentation tests differentiate those organisms sharply, *Bacillus chauvoei* strains consistently fermenting saccharose and failing to ferment salicin, while the reverse is true of *Vibrio septique*.

This *Vibrio septique* is a Gram-positive organism; it is motile in young cultures and in the exudate from infected animals. It presents a rather wide range of different forms according to the conditions of culture. In broth or in meat medium the organisms appear as rods of varying length somewhat more slender than *Bacillus welchii*. Spores are readily formed and are usually situated towards one extremity; central spores are, however, not

uncommon, while bulb-like types may be present, especially in young cultures. It is a strict anerobe. Surface colonies may be obtained on plates under good conditions or upon the surface of serum agar slants. The colonies are transparent and faintly opalescent, the contours smooth or indented, and the edges may be more or less crenated. The organism ferments glucose, levulose, galactose, maltose, lactose, and salicin but does not ferment saccharose, inulin, mannite or dulcite. Pigeons, guinea-pigs, mice, rabbits and dogs are all susceptible to infection with this organism, the most infective material being a 24 to 48 hour culture in glucose broth with or without a piece of fresh tissue.

c. *Bacillus Œdematiens*.

This organism was discovered by Weinberg and Séguin in 1915 and later identified by Legros, Vaucher, Dalyell and others. It is similar to the above organism and may, possibly, be identical with *Bacillus œdematis maligni* II, which was described by Novy. This is a Gram-positive organism, which is motile under strictly anaerobic conditions, but under a cover-slip is non-motile. In shape it is a stout rod as thick as *Bacillus welchii* but usually longer. The rods are frequently curved and the spores, which are readily formed in all media, are large and oval with slightly flattened ends. They are subterminal in position. The morphology must be studied in young cultures as autolysis sets in very early. Short chains and filaments are occasionally formed. Under strict anaerobic conditions, this organism grows well on most media, the surface colonies being flattened and tending to become confluent to a translucent film. It ferments glucose, levulose and maltose, but does not ferment galactose, saccharose, lactose, mannite, dulcite, inulin or salicin. The pathogenicity of different strains varies, but guinea-pigs, mice, rats, and rabbits are all susceptible.

d. *Bacillus Histolyticus*.

This organism is intermediate in position between the acutely pathogenic anaerobes discussed above and the secondary purely saprophytic types. It is a motile rod very frequently arranged in pairs. Very young cultures (14 to 16 hours) should be examined for this organism, as the bacilli degenerate very quickly in cultures and after 24 to 48 hours, many Gram-negative forms may be seen together with fusiform and skittle-shaped involution forms. The spores, which are easily formed in all media, are oval and usually sub-terminal and are considerably wider than the rod in which they arise. It is difficult to isolate, but it is found in wounds and has been obtained from earth. It ferments glucose, levulose and maltose. Strains of this organism vary greatly in pathogenicity. The bacillus is actively proteolytic and digests living tissue.

e. *Bacillus Fallax*.

This is a non-pathogenic type of anaerobe, but, like the *bacillus sporogenes*, seems to facilitate the growth of the true pathogens. It is a motile bacillus and is found in infected wounds and in cases of gas gangrene, occasionally being present in blood cultures from the patient. When recently isolated certain strains are pathogenic for guinea-pigs but this character is rapidly

lost by cultivation. It is a somewhat slender rod with rounded ends. It is often slightly curved. Gram-negative elements are frequent and there is rather a feeble capacity to retain the stain. Spores are not readily formed on any media but do occur in small numbers in meat and coagulated serum. These spores are oval and usually subterminal. This organism ferments glucose, levulose, and maltose (some strains act, also, on galactose).

f. *Bacillus Sporogenes*.

The less directly pathogenic anaerobes which proliferate in wounds make up a large group. Heller has studied a large number of these and has attempted to classify the rather disorganized group. Many of these organisms are truly ancillary to the condition of gas gangrene. Blood cultures not infrequently show the presence of these types. In mixed cultures they are favorable for the most part, although not invariably, to each other's growth as well as to that of the truly pathogenic forms. Apparently it is not till 2 or 3 days after the infliction of the wound that this type of anaerobe arises, while the pathogenic type may produce death rapidly. Pathogenic anaerobes may proliferate as apparently harmless saprophytes, but their presence is always to be regarded as a source of great potential danger.

This organism was first described by Metchnikoff in 1908, who distinguished 2 varieties *A* derived from the feces of healthy persons and *B* obtained from subjects suffering with chronic colitis. The bacillus sporogenes found in wounds appears to belong to Type *A*. It is present in a very large number of wounds and appears in acute cases of gas gangrene as well as in conditions in which the wound is progressing in a satisfactory manner. It is frequently found in earth and in practically all materials exposed to dust. It is a common inhabitant of the intestinal tract of man and animals and may be cultivated from a great variety of sources. It is very resistant, surviving as long as 8 days in 5 per cent. phenol or exposure in a capillary tube to 100°C. for 45 minutes. It is an actively motile rod, closely resembling the vibron septique in appearance and is more slender than the bacillus welchii. Spores are very readily formed in all media, are oval in shape and central or sub-terminal in position. It is Gram-positive, but in old cultures Gram-negative forms may be seen. It apparently ferments only glucose, levulose and maltose. In general cultures of bacillus sporogenes are not lethal to laboratory animals but some strains are capable of prolonging a putrid perforating gangrene of the leg injected and a small number of strains are described as being lethal in doses of about 1 c.c. and upwards. The presence of this organism in combination with *Bacillus welchii* definitely enhances the pathogenicity of the latter and produces a mixed putrefying type of gangrene. The influence on vibron septique is less certain but the general effect is the same.

The above are the more important of the pathogenic and ancillary anaerobes found in infected wounds, but there are a number of others which appear under certain conditions. Among these are found *Bacillus parasporogenes*, *Bacillus tertius*, *Bacillus cochlearius*, *Bacillus tetanomorphus*, *Bacillus aerofetidus*, *Bacillus bifermentans*, *Bacillus putrificus*, *Bacillus*

sphenoides, *Bacillus butyricus*, and *Bacillus multifерmentans tenalbus*. For a description of these types see Special Reports No. 39, Med. Res. Comm., 1919, from which much of the above discussion has been taken.

g. *Bacillus Tetani*.

This organism was described in 1884 by Nicolaier and cultivated by Kitasato in 1889. It is extremely difficult to isolate in a pure state, many of the cultures being found to contain other anaerobes. These contaminating organisms usually belong to the type of *Bacillus sporogenes*, but others are also found. This organism is a motile Gram-positive rod. In young cultures it is a rather stout bacillus about 4 to 8 microns in length and 0.4 to 0.6 microns in breadth. The spores are spherical and always strictly terminal, giving the familiar "drumstick" appearance. These spores are not formed in the ordinary culture media until the 3rd or 4th day. While this organism is classed as an obligate anaerobe, yet it may be made to grow under aerobic conditions by gradually increasing the oxygen in cultures, such habituation, however, to aerobic conditions usually resulting in loss or diminution in pathogenicity. It apparently does not ferment any of the following carbohydrates: Glucose, levulose, galactose, maltose, saccharose, lactose, inulin, starch, mannite, dulcitol or salicin. The tetanus bacillus has been found in the superficial layers of the soil and the earth of cultivated and manured fields seems to harbor the organism very frequently, due probably to its presence in the dejecta of the domestic animals. The pathogenicity of this organism depends entirely upon the soluble toxin which it produces.

h. *Bacillus Botulinus*.

This bacillus was first described by van Ermengem in 1896 in connection with an epidemic of meat poisoning in Flanders. It has not been found in wounds, and so far as is known is in no way involved in any aspect of the gas gangrene question. However, it has recently come into prominence in connection with cases of poisoning from various types of canned foods, especially olives and vegetables. *Bacillus botulinus* is a large straight rod with rounded ends, is 4 to 6 microns in length and 0.9 to 1.2 microns in thickness. It is somewhat larger than the vibrios septique but resembles it in general appearance. The bacilli are usually single but short filaments are not infrequently seen. Spores are not easily produced, although in suitable media such as glucose-gelatin of a distinctly alkaline reaction they do appear. They are small and oval and usually subterminal in position. The bacillus is motile and is Gram-positive, although care must be taken in the decolorizing with alcohol as the result may be doubtful if the alcohol acts too long. It is a strict anaerobe, but grows under proper conditions on the usual meat infusion media, especially at room temperature. This organism ferments glucose, maltose, lactose and starch but does not ferment galactose, saccharose, inulin, mannite, dulcitol or salicin. Botulism, as seen in the human, is due to the ingestion of infected meat, vegetables, olives, etc. It produces disease by means of a strong soluble toxin secreted by it. This is active in man when introduced through the gastrointestinal tract. By means of toxin-antitoxin studies Dickson has succeeded in differentiating

at least two types of this organism. Chickens appear to be very susceptible to Type *A* toxin and highly refractory to Type *B*.¹ Orr has recently shown that the feeding or injection of massive quantities of toxin-free spores of *B. botulinus*, may produce botulism, but believes that botulism poisoning in man, due to ingestion of spores, is probably very rare if it occurs at all.

8. Cholera Vibrio (Koch's Comma Bacillus).

This organism has been briefly discussed on page 138. It appears in stained preparations as a small, short slightly curved rod, which has led to the name of "comma" bacillus. It is about $2\ \mu$ long and $0.5\ \mu$ broad. It is not infrequent to observe long, straight involution forms, especially from liquefied gelatin cultures. This bacillus has a single flagellum at one end and shows very active motility. It stains easily with the ordinary stains and is Gram-negative.

The spirillum of cholera grows well on practically all media at room temperature, with the exception of potato.² The medium must be alkaline in reaction to insure the growth. This alkalinity is obtained by adding 3 c.c. of 10 per cent. sodium hydrate to 100 c.c. of neutral medium.

The growth on gelatin plates at room temperature is more or less characteristic. These colonies appear, after 24 hours, as round and regular white points, which later become irregular and show a granular appearance as if the colony were covered with ground glass. Within 48 hours liquefaction begins, the colony sinking into the depression. On gelatin stab-cultures, the liquefaction begins as a cup-shaped depression at the surface, a bubble of air being retained in the depression. The later liquefaction takes the form of a funnel, being much broader at the surface. Later the medium is entirely liquefied. Some of the vibrios resembling the cholera bacillus, as especially the Finkler-Prior vibrio, produce a more rapid liquefaction of the gelatin and do not show the bubble of air which is practically always present in the cholera cultures. This point is not, however, distinctive enough for strict diagnostic purposes. It is to be remembered, in this connection, that the other types of organisms found in the intestinal canal do not liquefy gelatin media.

On agar plates the colonies appear as pale, flat, thin growths which are transparent and show, by transmitted light, a peculiar opalescence. As the cholera spirillum is a strongly aerobic organism, we find a copious surface growth in 1 per cent. peptone solution containing $\frac{1}{2}$ per cent. sodium chlorid. Other organisms do not develop readily in this medium, so that we not infrequently may obtain an almost pure culture by use of this simple medium. The addition of a few drops of concentrated sulphuric acid to such a culture

¹ See Dickson, Monograph 8 of Rockefeller Institute, 1918; Graham and Brueckner, Jour. Bacteriol., 1919, IV, 1; Shippen, Arch. Int. Med., 1919, XXIII, 346; Edmondson and Giltner, Jour. Am. Med. Assoc., 1919, LXXIII, 907; Armstrong, Story and Scott, Pub. Health Rep., 1919, XXXIV, 2877; Emerson and Collins, Jour. Lab. & Clin. Med., 1920, V, 559; Sisco, Jour. Am. Med. Assoc., 1920, LXXIV, 516; Dickson and Howitt, Ibid., 718; DeBord, Edmondson and Thom, Ibid., 1220; Randell, Ibid., LXXV, 33; Graham and Schwarze, Ibid., 1921, LXXVI, 1743; Dickson, Ibid., LXXVII, 483; Koser, Edmondson and Giltner, Ibid., 1250; Nevin, Jour. Inf. Dis., 1921, XXVIII, 226; Reddish, Ibid., XXIX, 120; Semerak, Ibid., 190; Orr, Ibid., 1922, XXX, 118.

² See Teague and Travis, Jour. Infect. Dis., 1916, XVIII, 60r.

produces a burgundy-red color, due to the formation of nitroso-indol. Thus the cholera spirillum produces indol and, at the same time, reduces the nitrates of the culture medium to nitrites. While the colon bacillus produces indol, it is necessary to add a trace of nitrite before the nitroso-indol reaction is obtained. This *cholera-red reaction*, as it is called, was supposed to be distinctive for the cholera vibrio but it has been shown that other cholera-like organisms also react. A negative result with this test is, therefore, of greater value than a positive one.

In the bacteriological diagnosis of cholera, several points must be borne in mind. Select some of the "rice particles" and inoculate a peptone water culture as above, or plant 1 c.c. of fecal material in 50 c.c. of this medium. From this surface growth, at the end of six to eight hours at 37°C., sub-cultures are made upon other media and smears are examined and studied for the characteristic organisms. Glycerin and agar plates are inoculated and incubated for 24 to 48 hours, the former at room temperature and the latter in the incubator. It should be a rule to inoculate *Dieudonné's alkali-blood-agar* at the same time. This medium is prepared by mixing equal parts of defibrinated beef blood and normal (4 per cent.) sodium hydrate; heat in autoclave for one-half to three-fourths hour. Add 30 parts of this mixture, while still hot, to 70 parts of neutral 3 per cent. agar. Mix and sterilize in the autoclave. When cooled, pour into Petri plates. Allow the plates to dry by standing open in incubator for several hours and do not use these plates earlier than 24 hours after preparing. Colon bacilli and other extraneous organisms do not grow on this medium, while the cholera organism develops rapidly. The colonies are large, circular, crystal-clear by transmitted light and by reflected light gray. Microscopic examination of the organisms from these colonies shows many degenerative forms, very rarely any typical comma forms.

After these cultures are prepared test the motility of the organism in hanging drop and follow this by the agglutinating reactions and, especially, by the application of *Pfeiffer's bacteriolytic test*. This latter test is as follows: An emulsion of the organisms in question is injected into the peritoneal cavity of a guinea-pig, which has been immunized against a definite species of the vibrio. In 20 to 30 minutes remove, with a trocar, some of the peritoneal fluid and examine under the microscope. The organisms present will be non-motile and appear as more or less granular spherical masses instead of the typical forms, if the reaction is positive; if negative, the organisms will appear as in the usual preparations.¹

9. Gram-positive Cocci.

A. *Pneumococcus* (*Diplococcus* of Fraenkel; *Streptococcus Pneumoniæ*).

This organism is practically always found in the normal throat, so that its presence therein does not indicate a pathologic process, unless, the determination of the type of organism present shows that this is the actual etiologic factor in the existing pneumonia. It is found, also, as the primary etiologic

¹ See "The Bacteriological Diagnosis of Cholera," Pub. Health Rep., 1912, XXVII, 371; also, Kolle and Schürmann, *Kolle and Wassermann's Handb.*, 1912, IV, 1; Kabeshima, *Centralbl. f. Bakteriol.*, Abt. 1, 1913, LXX, 202; Craster, *Jour. Exper. Med.*, 1914, XIX, 581.

factor, in a wide range of infectious processes, so that it must not be regarded as limited to pneumonic types of infection. The morphology, staining characteristics and methods of grouping of this organism have been given on page 26, while its cultural peculiarities, especially, have been discussed on page 692. It is to be remembered that, from the morphological standpoint, it is oftentimes impossible to distinguish the pneumococcus from the streptococcus, as the former not infrequently appears in chains, and the typical lanceolate diplococci give place to forms which are more nearly true coccoid types.

Cultural methods must, therefore, be employed and the special peculiarities of the pneumococcus and streptococcus differentiated by the use of the blood-agar plates, the blood bouillon tubes, the use of bile acids, and the fermentative test with inulin as outlined on page 693. The agglutination test should, also, be employed with specific immune serum in all cases of doubtful diagnosis.¹

It is to be remembered that there are distinct varieties of pneumococci, which are distinguishable from one another only by careful study of the cultural peculiarities as well as by observation of their effects on animals. Transition forms from the pneumococcus to the streptococcus and *vice versa* are observed under certain cultural conditions, so that it is often a more than difficult problem properly to classify these organisms. One of these organisms, the *streptococcus mucosus capsulatus*, is probably to be considered with the true pneumococci. It occurs as round, or very slightly lance-shaped cocci, which are encapsulated, the capsule being wider than that of the pneumococcus. The organisms usually appear in pairs, but there are always seen a few in chains of four to eight organisms. The colonies on blood agar have a mucoid slimy consistency and have a greater tendency to become confluent than do those of the true pneumococcus.

B. Streptococci.

There are several varieties of streptococci, which are not readily distinguished by their morphology but which cultural peculiarities usually suffice to identify. These cultural points have been discussed on pages 694 and 695. It is to be said, however, that this differentiation is not a simple proposition, as these varieties readily change, the one into the other and, also, into forms which are hardly distinguishable from the typical pneumococcus. The chief types of the streptococcus are

a. *Streptococcus pyogenes (hemolyticus)*, a long-chained form which produces no green coloration on blood agar but shows a large colorless hemolytic area about its colonies.

b. *Streptococcus viridans*, a short-chain type producing green colonies on blood agar but only slight hemolysis.

c. *Streptococcus mucosus*, an encapsulated organism discussed above, producing no green colonies nor any hemolysis.

¹ See Dochez and Gillespie, Jour. Am. Med. Assn., 1913, LXI, 727; also, Floyd and Wolbach, Jour. Med. Research, 1913, XXIX, 493; Dochez and Avery, Jour. Exper. Med., 1915, XXI, 114; Lysill, Ibid., 146; Nicolle and Debaius, Bull. Acad. de Méd., 1910, LXXXI, 843; Avery and Cullen, Jour. Exp. Med., 1920, XXXII, 547; Lord and Nye, Ibid., 1922, XXXV, 658, 689, 699 and 703.

The standard routine method,¹ proposed by a special committee, for the isolation and identification of hemolytic streptococci from throats, sputa, and pathologic exudates, is very precise and clear-cut. This method involves the isolation of the hemolytic streptococcus into bouillon from a blood agar plate and the use of the bouillon culture for the following tests: (1) A study of the staining characteristics of the organism and the length, size, shape and arrangement of the cocci in the chains; (2) study of the ability of the organisms to lysis the corpuscles of a rabbit. Five-tenth c.c. of the bouillon culture is mixed with 0.5 c.c. of a 5 per cent. suspension of washed rabbit corpuscles in physiologic salt solution and incubated in a water-bath at 37°C. for 2 hours. Streptococci which lysis the blood completely in this time are to be regarded with suspicion; (3) to about 1 c.c. of bouillon culture add $\frac{1}{5}$ volume of sterile ox bile. Observe for 1 hour at room or incubator temperature. Solubility in bile, serves to distinguish certain strains of pneumococcus from the streptococcus, which does not dissolve in the bile; (4) fermentation reactions toward lactose, mannite, salicin, inulin, raffinose, and saccharose should be determined; (5) a study of the power of the organism to coagulate milk should be made, incubation being continued for 7 days; finally, (6) a study of the effects of inoculation of rabbits or mice should be made, the rabbits being inoculated intravenously and the mice intraabdominally.

Many other types have been isolated in pathological conditions and their exact classification is rather difficult.² Usually the organisms have shown characteristics which place them in classes intermediate between those given above and even have indicated their close relationship to the pneumococcus. One of these virulent organisms has been discussed on page 45, being called the *streptococcus epidemicus* and causing streptococcic sore-throat.

In these later days much work has been done on this group of organisms and much of value has been forthcoming. Some of this work is so important that the writer must discuss it briefly, in order properly to correlate the subject of streptococci to various pathological processes.

¹ Holman, Avery, Kinsella, and Brown, Jour. Lab. and Clin. Med., 1918, III, 618.

² Ulrich, Jour. Lancet, 1915, XXXV, 627; Rosenow, Jour. A. M. A., 1915, LXV, 1687; Ibid., 1916, LXVII, 662; Detweiler and Robinson, Ibid., 1933; Blake, Jour. Exper. Med., 1916, XXIV, 315; Holman, Jour. Med. Res., 1916, XXXIV, 377; Becker, Jour. Infect. Dis., 1916, XIX, 754; Krumwiede and Valentine, Ibid., 760; Smillie, Ibid., 1917, XX, 45; Blake, Jour. Med. Res., 1917, XXXVI, 99; Holman, Am. Jour. Med. Sc., 1917, CLIII, 427; Kinsella and Swift, Jour. Exper. Med., 1917, XXV, 877; Ibid., 1918, XXVIII, 169 and 181; Pilot and Davis, Jour. Infect. Dis., 1919, XXIV, 386; Nakayama, Ibid., 489; Bryan, Ann. Otol. Rhinol. and Laryngol., 1919, XXVIII, 337; Nichols, Ibid., 344; Avery, Dochez and Lancefield, Ibid., 350; Russell, Ibid., 374; Tongs, Jour. A. M. A., 1919, LXXIII, 1277; Dochez, Avery and Lancefield, Jour. Exp. Med., 1919, XXX, 179; Bliss, Bull. Johns Hopk. Hosp., 1920, XXXI, 173; Lewis, Boston Med. & Surg. Jour., 1920, CLXXXII, 240; Winslow, Rothenberg, and Parsons, Jour. Bact., 1920, V, 145; Tunnichliff, Jour. Am. Med. Assoc., 1920, LXXXIV, 1386; Jones, Jour. Exp. Med., 1920, XXXII, 273; Beckman, Deutsch. med. Wchnschr., 1920, XLVI, 1218; Tunnichliff, Jour. Am. Med. Assoc., 1920, LXXV, 1339; Arnold, Jour. Lab. & Clin. Med., 1920, VI, 587 and 591; Conner, Ibid., 767; Clawson, Jour. Inf. Dis., 1920, XXVI, 93; Jones, Ibid., 160; Clawson, Ibid., XXVII, 368; Haver and Frost, Ibid., 1921, XXVIII, 270; Tunnichliff, Ibid., XXIX, 91; Arnold, Jour. Lab. & Clin. Med., 1921, VI, 312; Havens and Taylor, Am. Jour. Hyg., 1921, I, 192; Foster, Jour. Bact., 1921, VI, 161 and 211; Norton, Rogers, and Georgieff, Jour. Am. Med. Assoc., 1921, LXXVI, 1003; Bumpus and Meslier, Arch. Int. Med., 1921, XXVII, 326; Herrold, Jour. Inf. Dis., 1922, XXX, 80; Hodge and Cohen, Ibid., 400.

Davis¹ has called attention to the close association of streptococcic infections to chronic arthritic troubles and finds that the atrium of infection in these arthritides is usually the tonsil or peritonsillar structures. As is demonstrated the streptococcus hemolyticus, the diplococcus (streptococcus) rheumaticus and the streptococcus epidemicus (see p. 45) are very prone to attack the joints; while the streptococcus viridans rarely does so. Such cases can be separated by the clinical findings and history of tonsillitis in the patients from the other types of deforming arthritides. The bacteriologic examination of cultures from the tonsil clears up the etiology. In taking the cultures from the tonsil, the material must be obtained from the depths of the crypts after the tonsil has been incised. In such cultures the streptococcus hemolyticus is usually found. The streptococcus viridans is found in surface cultures, but this is disregarded as it is less virulent and is rarely associated with arthritic involvements. It is to be recalled that the streptococcus hemolyticus may be found on the surface of the tonsils in ordinary streptococcic tonsillitis, and even on normal tonsils, but these do not necessarily have any special general value in the absence of clinical history of joint troubles. In the treatment of these cases of arthritis due to streptococcic infection, proper vaccines often work like magic. Two hundred million of the killed organisms are given at a dose with five-day intervals between the administrations.

Rosenow² in his work on the pneumococcus and streptococcus has isolated certain organisms from infected joints, blood cultures and tonsillar infections in cases of acute articular rheumatism and finds organisms corresponding to the micrococcus rheumaticus of Poynton and Payne. He finds, however, that this organism resembles very closely the streptococci, its virulence being less than that of the streptococcus hemolyticus and greater than that of the streptococcus viridans. By animal passage and other means three of the strains isolated from acute rheumatism have been converted into typical hemolytic streptococci on the one hand and into pneumococci on the other. The cultures made from the involved joints and regional lymph nodes have revealed three types of streptococci. One produces green colonies on blood agar and forms long chains; a second produces a narrow zone of hemolysis and forms short chains; while the third forms a grayish-brown colony without affecting perceptibly the blood in the medium. This latter organism appears as a diplococcus, in short chains and as single cocci. The most distinctive cultural feature of these types is their production of a very high acid reaction in dextrose-broth and abundant growth at low temperatures. Progressive transmutation from a streptococcic type to a pneumococcus is easily shown.

¹ Jour. Infect. Dis., 1912, X, 148; Jour. Am. Med. Assn., 1913, LXI, 724. See, also, Richards, *Ibid.*, 1914, LXII, 110; Holman, Jour. Path. and Bacteriol., 1915, XIX, 478.

² Jour. Am. Med. Assn., 1913, LX, 1233; Jour. Infect. Dis., 1914, XIV, 1 and 61; Rosenow, New York Med. Jour., 1914, XCIX, 270; Jour. Am. Med. Assn., 1914, LXII, 1146; *Ibid.*, 1914, LXIII, 903; Davis, *Ill. Med. Jour.*, 1914, XXVI, 158; Billings, *Ibid.*, 164; Jour. Am. Med. Assn., 1914, LXIII, 899; Smith and Brown, Jour. Med. Research, 1914, XXXI, 455; Hopkins and Lang, Jour. Infect. Dis., 1914, XV, 63; Thro, *Ibid.*, 34; Holman,

Billings,¹ on the basis of this work of Davis and Rosenow, has studied the clinical aspects of arthritic cases and finds that a chronic focal infection is associated with many of these arthritides. Clinically, he finds the focal disease in the faucial tonsil, dental alveoli or jaw, antra of head, etc., in a large number of cases. In other cases chronic colon, gonorrheal or streptococcic infection of the seminal vesicles and prostate were found to be the focal point of the arthritic involvement. Cultures were prepared from the material obtained from the focal infection, sub-cultures made and the dominant organism isolated in pure culture. From these cultures vaccines were prepared and used with excellent results. Billings is especially emphatic upon the point of using the auto-vaccine from the dominant isolated organism and believes that only by this method may good result from vaccine treatment.

C. *Staphylococcus Pyogenes*.

These organisms grow well on all types of media. Their cultures on agar are best adapted to the development of the pigment, which is the characteristic differentiating point of members of this species. Cultivation at room temperature frequently brings out the pigment better than growth in the incubator. These organisms are readily recognized, in stained preparations, by their morphology. They appear as distinct cocci, five to fifty in a cluster often compared to a bunch of grapes.² Rarely do they occur singly or in pairs. Occasionally short chains may be observed, but most of them are clumped. In purulent material these organisms may be both intra- and extracellular, but their staining characteristics readily distinguish them from the Gram-negative types of organism.

10. Gram-negative Cocci.

A. *Gonococcus* (*Diplococcus* of Neisser).

The morphological and staining characteristics of the gonococcus have been discussed on page 795. It is to be emphasized that this organism cannot, in every case, be distinguished by its morphology, staining characteristics and intracellular position from other organisms, especially from the meningococcus, micrococcus catarrhalis and a few saprophytic diplococci, such as diplococci flavus, crassus and cinereus.³ In judging of the possibility of a certain organism being the gonococcus, the origin of the culture must be known and something of the clinical history must be given. Further, it is to be recalled that the intracellular grouping of the organisms is not especially limited to the gonococcus nor is it always present in every gonorrheal case. Such arrangement is given both by the meningococcus and micrococcus catarrhalis. In the early cases of gonorrhea, when pus cells are very few in number, the gonococci are for the most part extracellular, some of them lying upon the epithelial cells, but practically none being found within the pus cells.

Ibid., 293; Crabtree, Ibid., 309; Davis, Ibid., 378; Rosenow, Ibid., 1915, XVI, 367; Thro., Ibid., 1915, XVII, 227; Broadhurst, Ibid., 277; Brawley, Ill. Med. Jour., 1915, XXVIII, 178.

¹ Arch. Int. Med., 1912, IX, 484; Jour. Am. Med. Assn., 1913, LXI, 819.

² See Walker and Adkinson, Jour. Med. Res., 1917, XXXV, 373.

³ See Kligler, Jour. Exper. Med., 1913, XVII, 653.

As the pus cells increase in number, the intracellular types become predominant owing to the active phagocytosis. In the later stages and in the chronic forms of infection, the extracellular types become more frequent, very few intracellular forms being observed.

As the gonococcus does not grow readily, if at all, on the ordinary culture media, special media is necessary. Albumin, preferably of human origin, is essential in any medium to be used. The best medium is Wertheim's serum agar prepared by adding 1 part of human serum to 2 to 3 parts of nutrient agar. The serum is warmed to 40°C. and added to the liquefied agar of the same temperature. Instead of the serum, ascitic or hydrocele fluid may be substituted as suggested by Kiefer and Steinschneider. In fact, ascitic agar is probably more frequently used than is the serum agar. Blood agar also affords an excellent medium for the development of the gonococcus.¹

As Wherry and Oliver have shown the gonococcus grows best at a lowered oxygen tension. Swartz and Davis have introduced a method for cultivation of the gonococcus, which depends on this principle and assures almost constant cultures of this organism. The medium, which they employ is a 2 per cent. beef or veal infusion agar, prepared in the usual manner and brought up to a reaction of P_H 7.6, phenolsulphonephthalein being used as an indicator. After autoclaving, the reaction comes to about P_H 7.4. Sterile ascitic, pleuritic or hydrocele fluid is added to the melted agar in the proportion of 1 part of fluid to 2 parts of agar. The tubes are then stoppered with sterile rubber stoppers and slanted. The tubes are then kept in the incubator, which detects any contamination. The inoculation of the slants is made as plentifully as possible. It is important to have the medium at body temperature when the inoculation is made. Immediately after inoculation, the tube, held horizontally, is turned so that the agar slant is uppermost. Held by the butt, it is then passed longitudinally through the Bunsen flame about 3 times and quickly stoppered. The tubes are then placed in the incubator, when visible colonies appear in 12 to 15 hours and profuse growths in 24 hours. The viability of the gonococcus on this medium is about 7 days.

On the above media, colonies of the gonococcus appear in 24 hours as grayish-white points, which merge later into growths of a grayish-blue tinge with slight iridescence. These colonies have a slimy consistency, are round but soon become confluent. The gonococcus is very sensitive to drying, so that the culture medium must have plenty of water of condensation. It survives exposure to air for only a short time, although in masses of dried pus it may live, exceptionally, for six or seven weeks. In favorable culture media it rarely maintains its vitality more than 48 to 72 hours at room temperature but will live longer if kept in a refrigerator (Jordan).

¹ For a discussion of the Human plasma glucose-agar medium of Thomson, the Tryptamine-blood-extract agar and broth of Cole and the Trypsinized pea-extract agar of Gordon and Hine see Special Report No. 10, Medical Research Committee of National Health Insurance, London, 1918. See also, Wherry and Oliver, *Jour. Infect. Dis.*, 1916, XIX, 288; Watabiki; *Ibid.*, 733; Thomson, *Brit. Med. Jour.*, 1917, I, 869; Herrold, *Jour. Am. Med. Assoc.*, 1920, LXXIV, 1716; Swartz and Davis, *Ibid.*, LXXV, 1124; Davis and Swartz, *Jour. Inf. Dis.*, 1920, XXVII, 591; Jenkins, *Jour. Path. & Bact.*, 1921, XXIV, 160; Hermanies, *Jour. Inf. Dis.*, 1921, XXVIII, 133; Rockwell and McKhann, *Ibid.*, 249; Hermanies, *Ibid.*, XXIX, 11; Cook and Stafford, *Ibid.*, 561; Erickson and Albert, *Ibid.*, 1922, XXX, 268.

The gonococcus is bacteriologically identified by its characteristic morphology, its Gram-negative staining, its frequent intracellular position and by its ready growth on blood or ascitic agar and its lack of growth on ordinary media. These characteristics are, however, not sufficient to differentiate it from the closely related organisms, which will be discussed below. It is to be said that a diagnosis of a gonorrheal infection outside of the genital tract is never safe if based entirely on microscopic evidence, and the cultural evidence is not always clear. The micrococcus catarrhalis is not infrequently the etiologic factor in a genito-urinary infection as well as in other parts of the system, while the meningococcus very often causes certain genito-urinary complications, which may be associated with the presence of this latter organism in this tract (see Koch¹). Further, in chronic cases of true gonorrhea, it is very frequently a difficult matter to detect the gonococcus either microscopically or culturally. The organisms found may be Gram-variable and few will be intracellular, so that great care must be exercised in diagnosis. In such cases one should use the provocative test of administering some irritant or allowing the patient to partake freely of alcohol. Such a procedure followed by milking the prostate will usually result in the appearance of typical gonococci in the purulent material expressed. Of course in these cases one may use the complement-fixation test or the vaccination test as suggested by Irons, and later, by Fromstein (see p. 708).

From the clinical point it may tax the resources of the physician to establish a diagnosis of gonorrhea in any given case, outside of those cases of acute gonorrhea in which the history and symptomatology are clear. However, in cases of meningitis with genito-urinary complications or of general systemic gonorrheal infection with meningeal symptoms, the diagnosis is not so simple, as a differentiation must be established between the meningococcus and gonococcus.

In this differentiation, cultivate the material obtained from the urethral discharge or from lumbar puncture upon faintly alkaline ascitic agar for 24 to 48 hours. The meningococcus grows more luxuriantly than the gonococcus. The colonies of the former are larger (2 to 3 mm. in diameter) than those of the latter (1 to 1.5 mm.). On microscopic examination of these colonies with the low power, the colonies of the meningococcus are found to be flat, homogeneous, usually round, very slightly irregular border but never notched; while those of the gonococcus are irregular, border usually notched and center always elevated. On cultivating these organisms on litmus-ascitic-agar media containing various sugars, the gonococcus will show acid production in the dextrose media only; while the meningococcus produces acid in both dextrose and maltose, but in no others.

The micrococcus catarrhalis, with which the gonococcus may be often confused, grows well on ordinary media. It does not produce acid in any sugar-containing medium. Its morphology, staining characteristics and intracellular position are, however, exactly like those of the gonococcus.

B. Meningococcus (Diplococcus Intracellularis Meningitidis).

¹ Kolle and Wassermann's Handbuch, 1913, IV, 689.

The morphological and staining characteristics of this organism of Weichselbaum have been given on page 815. For the bacteriological diagnosis of a meningococcic infection, one may use spinal fluid, blood, or purulent products from metastatic points, secretions of nose and bronchi. This material should be examined as soon as possible after obtaining, as the meningococci are not especially resistant to various changes.

For the development of this organism, especially of the first generation, media are necessary which contain animal, preferably human, albumin. The blood agar or ascitic agar given above are the best to use. The cultural peculiarities of the meningococcus on this medium have been given.¹ From the cultures the organisms not infrequently appear variable in size and show variable depth of staining, although they are always Gram-negative.

In examining spinal fluid for these organisms, centrifuge the fluid in a sterile tube and plant two or three ascitic agar plates with the sediment. Isolate the colonies by sub-culture if necessary and identify the organism microscopically. Inoculate special sugar-containing media and differentiate by the results mentioned above. The meningococcus produces acid only in dextrose and maltose media.

In examining material from the nose or throat simple microscopical examination is of little avail, as we have the possibility of finding the micrococcus catarrhalis as well as the saprophytic diplococci previously mentioned. Cultural work and differentiation on sugar media are absolutely essential. These saprophytic cocci, especially the *diplococcus crassus*, are found in both the lumbar fluid and nasal secretions. This latter diplococcus produces acid in dextrose, maltose, galactose, levulose, cane-sugar and lactose media. This organism is probably identical with Jäger's meningococcus. Some of the varieties of the *diplococcus flavus* ferment levulose in addition to dextrose and maltose, while other types (*Diplococcus flavus* III) acts exactly like the men-

[+ = Acid; — = No acid.]

	Glucose	Maltose	Mannose		Glucose	Maltose	Mannose
<i>M. pharyngis</i>	+	+	+	<i>M. flavus</i> III...	+	+	+
<i>siccus</i>							
<i>M. flavus</i> I.....	+	+	+	Meningococcus.	+	+	—
<i>M. flavus</i> II.....	+	+	+	<i>M. catarrhalis</i> ..	—	—	—

ingococcus.² It will be seen, therefore, that the fermentation test should be applied in all differential work, where the meningococcus is concerned.

¹ See Cohen and Markle, Jour. A. M. A., 1916, LXVII, 1302; Douglas, Med. Jour. Australia, 1916, II, 382; Lloyd Jour. Path. and Bacteriol., 1916, XXI, 113; Gordon, Hine and Flack, Brit. Med. Jour., 1916, II, 678 and 682; Davison, Davison and Miller, Jour. Exper. Med., 1917, XXVI, 770; Bushnell, Jour. Med. Res., 1918, XXXVIII, 1. Binger (Jour. Infect. Dis., 1919, XXV, 277) has shown that methylene blue inhibits the growth of meningococci in fairly high dilutions.

² See Kutscher, Kolle and Wassermann's Handbuch, 1913, IV, 589. The meningococcus has been grouped, on the basis of immunological reactions, into 4 groups. See Ellis, Brit. Med. Jour., 1915, II, 881; Arkwright, Ibid., 885; Olmstead, Du Bois, Neal and Schweitzer, Jour. Immunol., 1916, I, 307; Tulloch, Jour. Royal Army Med. Corps., 1917, XXIX, 66; Vines, Jour. Path. and Bacteriol., 1918, XXII, 50; Fildes, Brit. Jour. Exp. Path., 1920, I, 44; Wadsworth, Gilbert and Hutton, Jour. Exp. Med., 1921, XXXIII, 90.

The method to be followed is as follows: The sugars to be selected are glucose, maltose and mannose. Ten per cent. solutions of these sugars in distilled water are prepared and sterilized in the autoclave at 15 pounds pressure for 15 minutes. One c.c. of the sugar solution is then added to 10 c.c. of blood or ascitic agar medium, with a reaction of 0.2 to 0.5, to which enough pure sterile litmus solution is added to give a blue tint. The tubes are then inoculated and placed in the incubator for 24 hours. The reaction of the Gram-negative organisms common to the naso-pharynx, and which are of importance in the differentiation as far as the determination of actual infection or carriers is concerned, is as given in the preceding table (as shown in Reprint 413, Public Health Reports, U. S. P. H. S.).

C. *Micrococcus Catarrhalis*.

This organism is discussed on page 17. It is found in catarrhal conditions of nose and throat and, also, in infections of the genito-urinary tract. It grows readily on all media. On ascitic agar plates the colonies are white, firm, irregular, dry and somewhat smaller than the growths of meningococcus. Under low microscopic power they appear brown and granular, while those of the meningococcus are yellow and homogeneous and of the gonococcus grayish and slimy. The firm and dry quality of the colony is characteristic of the micrococcus catarrhalis. From the ordinary staphylococci, it is differentiated by its non-liquefaction of gelatin.

Preparations made from culture media show involution forms, some of the organisms being very large. In the direct smears of purulent material the organisms are Gram-negative, intracellular or extracellular biscuit-shaped diplococci. For its identification cultural work is necessary and a study of its fermentative powers is essential. The micrococcus catarrhalis does not produce acid in any sugar-containing medium. An organism described as the *Micrococcus cinereus* is probably identical with this organism of catarrh.

VIII. VACCINES

In previous sections of this book the problems of immunity in its relation to opsonins, agglutinins and precipitins have been discussed briefly. It was shown that the injection of a vaccine (a suspension, in physiologic salt solution, of a certain number of killed organisms) into the body of a patient suffering with a chronic infection due to the organism injected brought about a systemic reaction by which the resistance of the patient was increased. In this way it was hoped that the natural resources of the body would be brought into activity by the stimulation of antibody formation and that this would result in great benefit to the patient. Further, by the use of vaccines, the resistance of the human organism against bacterial invasion could be so increased that immunity was conferred, for a variable period of time, toward the organism so injected. The most recent use of this latter principle is the antityphoid vaccination, which will be discussed later.

It will be remembered that patients, who have passed through an attack of an infectious disease, have acquired, in most infectious diseases, an immunity against reinfection. An attempt is often made, especially in the treatment of infections with diphtheria organisms, tetanus bacilli and meningococci, to aid the patient in his efforts to overcome the invasion, that is to help him in acquiring immunity, by injection of serum from an animal previously immunized against such infection. This serum or antitoxin supplies antibodies to the infected patient, with which he may overcome the toxic products of bacterial development in the system. In other words, serum therapy confers immunity, shown in recovery from the acute infection, by aiding the system through introduction of antibodies produced outside the system of the patient. Vaccine therapy, on the other hand, produces immunity by causing the production of antibodies within the system itself. It must be recalled that patients who have been inoculated with serum (protein) often become sensitized to its action to such an extent that a later injection of the same serum, even in much smaller dosage, may provoke serious and even fatal results. This is the phenomenon of *anaphylaxis* and must be remembered whenever one is dealing with the parenteral introduction of protein material into the system.¹

In this section the writer wishes to discuss the method of preparation of vaccines, both of the stock and autogenous varieties. The elaboration of the principles upon which their use is based as well as a discussion of the therapeutic value finds no place here.

I. Preparation of Vaccines.

A. Stock Vaccines.

The material for this work is obtained, under the strictest precautions to prevent contamination, from a focus of infection with the organism whose vaccine is desired. It is at once plated upon general or special medium, depending upon the organism in question, and the organism isolated in pure culture. This may be a simple matter or it may be a very complicated one.

¹ See Besredka, in Kraus and Levaditi's Handbuch, 1911, Ergänzungsbd. I, 209; Biedl and Kraus, *Ibid.*, 255; Abderhalden, *Ztschr. f. physiol. Chem.*, 1912, LXXXII, 109; Wells and Osborne, *Jour. Infect. Dis.*, 1913, XII, 341; Weil, *Jour. Med. Research*, 1913, XXVIII, 243; Auer and Van Slyke, *Jour. Exper. Med.*, 1913, XVIII, 210; Zinsser, *Ibid.*, 219; Vaughan, *Jour. Am. Med. Assn.*, 1913, LXI, 1761; Friedberger, *Nederl. Tijdschr. v. Geneesk.*, 1913, II, 1065 and 1278; Nixon, *Brit. Med. Jour.*, 1913, II, 1351; Weil, *Jour. Med. Research*, 1913, XXIX, 233; Leschke, *Ztschr. f. exper. Path. u. Therap.*, 1914, XV, 23; Seligmann, "Anaphylaxie," in *Handb. d. Biochem. des Menschen u. d. Tiere, Ergänzb.*, 1913, 248; Vaughan, "Anaphylaxis and Infection," *Harvey Lectures*, 1913-14, p. 132; Bradley and Sansum, *Jour. Biol. Chem.*, 1914, XVIII, 497; Wallace, *Jour. Am. Med. Assn.*, 1914, LXII, 1166; Longscope, *Jour. Exper. Med.*, 1915, XXII, 703; Soula, *Presse Méd.*, 1916, XXIV, 471; Weil, *Jour. Immunol.*, 1916, I, 1, 10, 35 and 47; Klinkert, *Nederl. Tijdschr. v. Geneesk.*, 1917, I, 202; Novy, DeKruif and Novy, *Jour. Infect. Dis.*, 1917, XX, 499; Novy and DeKruif, *Ibid.*, 536, 566, 580, 618, and 620; *Jour. A. M. A.*, 1917, LXVIII, 1524; Zinsser and Parker, *Jour. Exper. Med.*, 1917, XXVI, 411. Newer work along the line of Vaughan's Protein Poison includes the following: Underhill and Hendrix, *Jour. Biol. Chem.*, 1915, XXII, 405; Pryer, *Jour. Lab. and Clin. Med.*, 1916, I, 490; Cumming and Chambers, *Ibid.*, 428; Vaughan, *Ibid.*, 643 and 851; *Ibid.*, 1916, II, 15; *Jour. A. M. A.*, 1916, LXVII, 1550; Boughton, *Jour. Lab. & Clin. Med.*, 1920, V, 507; Dale, *Bull. Johns Hopk. Hosp.*, 1920, XXXI, 310; Flexner, *Science*, 1920, LII, 615; Manwaring, *Jour. Am. Med. Assoc.*, 1921, LXXVII, 849.

In any case, all the differential points previously given for the varying organisms must be kept in mind, so that proper medium and methods of isolation may be employed. It is absolutely essential that the final culture be pure, if the vaccine is to be of any value.

In order to obtain a large amount of the pure culture, transfers from the original pure culture are made to three or four tubes of the medium best suited for its development and these tubes incubated for 24 to 48 hours. The cultures are then washed from the surface of the medium, using sterile physiologic salt solution as the menstruum and scraping the surface lightly with the platinum needle to insure the removal of the organisms. Pour this suspension into a sterile flask or large tube and shake thoroughly to break up the bacterial masses. This is an important point in the technic, as otherwise the suspension will not be uniform. If necessary, one may filter out the large clumps by means of a sterile filtering arrangement.

Having prepared the suspension, this is then sterilized by heating in a water-bath to 60°C. for one hour. From this tentatively sterilized suspension, cultures are made upon proper media by transferring several loopsful of the suspension to the surface of the slanted media and incubating for 48 hours. If no growth occurs, the suspension may be regarded as sterile.¹

Standardization.—The sterilized vaccine is now examined for the number of bacteria per c.c. of the suspension. The method usually adopted is to compare the number of bacteria in a definite volume of suspension with the number of red blood cells in the same volume of freshly drawn blood. The technic is as follows. By means of a graduated capillary pipet place equal volumes of blood and of bacterial suspension on a glass slide, mix thoroughly and spread as for an ordinary blood slide. Stain with Wright's or other blood stain. Count the number of red cells and of bacteria in a number of microscopic fields and obtain an average of each. As the number of red cells per cu. mm. is arbitrarily fixed at 5 million, the relation of bacteria to the red cells, as shown by the counts, will readily give the number of bacteria in the cu. mm. The number in 1 c.c. is obviously 1,000 times greater.²

Having obtained the concentration of the original suspension, the vaccine must be standardized so that each c.c. shall contain a standard number of bacteria. This is done by diluting with sterile physiologic salt solution (which contains sufficient cresol to bring the concentration of the final product up to 0.2 to 0.4 per cent. cresol) until the proper strength is reached. The addition of the cresol prevents future contamination of the vaccine and is an additional safeguard as to sterility of the product. The standard maximum dosage per c.c. of some of the more usual stock vaccines is as follows:

¹ King and Davis (Am. Jour. Pub. Health, 1914, IV, 917) have shown that potassium tellurite is an excellent indicator of sterility. Characteristic black compounds are produced if a little of the salt be added to fluids containing viable bacteria.

² See Hopkins (Jour. Am. Med. Assn., 1913, LX, 1615) for a method of standardizing vaccines based on centrifugation and determination of percentage of bacterial sediment; Glynn, Powell, Rees and Cox, Jour. Path. and Bacteriol., 1914, XVIII, 379; Blaker, Indian Jour. Med. Research, 1914, I, 726; Harvey and Acton, Ibid., 1914, II, 648; Fitch, Jour. Am. Med. Assoc., 1915, LXIV, 893. Dreyer and Gardner, Biochem. Jour., 1916, X, 399; Gates, Jour. Exper. Med., 1920, XXXI, 105.

Staphylococcus,	500 million
Streptococcus,	100 million
Colon bacillus,	100 million
Gonococcus,	100 million
Typhoid,	1,000 million
Pneumococcus,	100 million

Of course this maximum is not suited to all cases. The dosage must be varied for the individual and for the type of infection.

B. Mixed Stock Vaccines.

A prerequisite to the use of any vaccine should be the bacteriological identification of the infection in question. The use of a simple or mixed stock vaccine is never justified without such a precaution. It is questionable whether one should ever use a mixed stock vaccine, but if the bacteriological examination has revealed a mixed infection, a proper mixture may be used in the proportions suitable for each separate constituent of the vaccine. In recent years the number and varieties of mixed stock vaccines have increased to such an extent that they are to be regarded as questionable therapeutic agents. To quote from Billings, "There is a value in vaccine therapy but to obtain benefit from vaccines, they should be rationally used. To use vaccines, simple or mixed, in the treatment of a patient, without first ascertaining the nature of the disease, and, if infectious, the kind of invading organism, is unscientific, reprehensible and wrong. When the mixed vaccines or the filtrate of culture-broth of countless organisms are used, it is like the shotgun prescription of our ancestors of the profession. They were and are intended to hit something." Such usage must be regarded, therefore, as mere guesswork.

C. Autogenous Vaccines.

These are the proper agents to use whenever a vaccine is to be employed. The specific toxins of the organism call into activity the specific antibodies, which are necessary to overcome the effects of the organism causing the infection, for which the autogenous vaccine is being prepared. "Instead of adding a heavier burden to the individual's immunizing mechanism, already taxed in its struggle against the invading bacteria, these bacteria, reintroduced as autogenous vaccines, if successful stimulate further therapeutic immunizing responses." It can be considered as established that these autogenous vaccines are the ones giving the best results.

These autovaccines are prepared from the discharges, excretions, blood, etc., of the patient. The effort to obtain pure cultures and definitely to identify the organism or organisms which are dominant must be painstaking, both aerobic and anaerobic cultures being made in many cases. If there be a mixed infection, each organism is isolated in pure culture and the suspensions of the pure cultures are then mixed in such a way that the resulting combination will contain the organisms in their usual strength per c.c. In this standardization of the autogenous vaccines a little more latitude is allowed than in the case of stock vaccines, as the dosage of the autovaccine

must be adjusted more to the individual than to the standard. In other words, one must use these autovaccines in graduated dosage, beginning with a small dosage and working up.

It is true that occasionally mixed autovaccines are prepared which have not been sufficiently differentiated. This method is uncertain and crude. The method employed is to obtain a culture of all the organisms present in the discharge and prepare a suspension which shall have a standardized content within the range of usefulness of the organisms present. Here we are confronted with the same proposition as in the case of mixed stock vaccines. All the organisms should be identified, if not they should not be used. In this way a more reliable and more scientific product will be furnished.¹

2. Antityphoid Vaccination.

The basis of the use of vaccines, as a preventive immunizing inoculation against typhoid fever, is the observation that one attack usually confers immunity from further attacks. This use of vaccines has become widespread and is promising great results, especially in those subjected to variations in the sanitary surroundings. The doses are given at ten-day intervals; the first dose contains 500 million organisms and the second and third doses 1 billion bacteria each. A local reaction is usually present but this subsides within 48 hours, while a general systemic reaction is not always observed. As this measure is of such importance to health and sanitary officers, as well as to physicians in general, I give, herewith, some references for consultation.²

¹ See Klenk, *Interstate Med. Jour.*, 1915, XXII, 1209; Warden, *Jour. A. M. A.*, 1915, LXV, 2080; Babcock, *Lancet-Clinic*, 1916, CXV, 139; Hekman, *Nederl. Tijdschr. v. Geneesk.*, 1916, I, 2157; Hess, *Am. Jour. Dis. Child.*, 1916, XII, 466; Wohl, *Am. Jour. Med. Sc.*, 1916, CLII, 262; Hektoen, *Jour. A. M. A.*, 1916, LXVI, 1591; Fox, *Ibid.*, 2064; Sewall Mitchell, and Powell, *Ibid.*, 1916, LXVII, 95; Satterlee, *Ibid.*, 1729; Davis, *Ibid.*, 1917, LXVIII, 159; Sholly, Blum, and Smith, *Ibid.*, 1451; Luttinger, *Ibid.*, 1461; Rosenow and Osterberg, *Ibid.*, 1919, LXXIII, 87; Moody, *Jour. Am. Med. Assoc.*, 1920, LXXIV, 391; Flexner, *Ibid.*, 1921, LXXVI, 33; Geraghty, *Ibid.*, 35; Blackfan, *Ibid.*, 36; Flexner, *Ibid.*, 108; Park, *Ibid.*, 109; Amoss, *Ibid.*, 110; Cole, *Ibid.*, 111; Nicoll, *Ibid.*, 112; Engman, *Ibid.*, 176; Russell and Nichols, *Ibid.*, 177; Cecil, *Ibid.*, 178; Stimson, *Ibid.*, 241; Davison, *Ibid.*, 242; Teague, *Ibid.*, 243; Gay, *Ibid.*, 244; Miller, *Ibid.*, 308; Cowie, *Ibid.*, 310; Culver, *Ibid.*, 311; Petersen, *Ibid.*, 312.

² See Wright, *Lancet*, 1896, II, 807; Brit. Med. Jour., 1897, I, 16; Leishman, *Jour. Roy. Inst. Pub. Health*, 1910, XVIII, 394; Firth, *Jour. Roy. Army Med. Corps*, 1911, XVII, 495; Russell, Boston, *Med. and Surg. Jour.*, 1911, CLXIV, 1; Nelson and Hall, *Jour. Am. Med. Assn.*, 1911, LVII, 1759; Schereschewsky, *Pub. Health Rep.*, 1911, XXVI, 1508; Davis, *Jour. Am. Med. Assn.*, 1912, LVIII, 537; Russell, *Ibid.*, 1331; Maverick, *Ibid.*, 1672; Engelbach, *Interstate Med. Jour.*, 1912, XIX, 537; Albert and Mendenhall, *Am. Jour. Med. Sc.*, 1912, CXLIII, 232; Williams, *Ibid.*, 352; Callison, *Ibid.*, 1912, CXLIV, 350; Russell, *Bull. 2, War Dept. Office Surg. Gen., Washington*, 1913, 7; Craig, *Ibid.*, 15; Nichols, *Ibid.*, 23; Hunt, *Am. Jour. Med. Sc.*, 1913, CXLV, 826; Hiss, *Jour. Med. Research*, 1913, XXVIII, 385; Russell, *Jour. Am. Med. Assn.*, 1913, LXI, 666; Metchnikoff and Besredka, *Ann. de l'Inst. Pasteur*, 1913, XXVII, 597; Russell, *Am. Jour. Med. Sc.*, 1913, CXLVI, 803; Foxworthy, *Military Surg.*, 1914, XXXIV, 332; Sachs, *Med. Klin.*, 1914, X, 1538; Russell, *Jour. Am. Med. Assn.*, 1914, LXII, 1371; Krumbhaar and Richardson, *Am. Jour. Med. Sc.*, 1915, CXLIX, 406; Thomsen, *Hospitalstid.*, 1915, LVIII, 211; Stursberg and Klose, *Münch. med. Wehnschr.*, 1915, LXII, 380; Cecil, *Jour. Infect. Dis.*, 1915, XVI, 26; Biedl, *Wien. klin. Wehnschr.*, 1915, XXVII, 125; Paltauf, *Ibid.*, 125; Kirschbaum, *Ibid.*, 208; Eggerth, *Ibid.*, 209; Csernel, Marton and Feistmantel, *Ibid.*, 220; Sladek and St. Kotlowski, *Ibid.*, 389; Goldscheider and Aust, *Deutsch. med. Wehnschr.*, 1915, XLI, 361; Kisskalt, *Ibid.*, 303; Schneider, *Ibid.*, 393; Reibmayr, *Münch. med. Wehnschr.*, 1915, LXII 610; Rhein, *Ibid.*, 427; Bourke, Evans and Rowland, *Brit. Med. Jour.*, 1915, I, 584; Widai, *Bull. de l'Acad. Med.*, 1915, LXXIV, 205; Vincent and Chantemesse, *Ibid.*, 225; Harris and Ogan, *Jour. Am. Med. Assn.*, 1915, LXIV, 3; Garbat,

Ibid., 489; Trowbridge, Finkle and Barnard, Ibid., 728; Elmer, Ibid., 1147; Lyster, Ibid., 1915, LXV, 510; Jobling and Petersen, Ibid., 515; Sawyer, Ibid., 1413; Schottstaedt, Ibid., 1713; Nichols, Jour. Exper. Med., 1915, XXII, 780; Holler, Ztschr. klin. Med., 1915, LXXXI, 462; Noack, Ibid., LXXXII, 132; Stieve, Deutsch. Arch. f. klin. Med., 1915, CXVII, 462; Mertz, Ztschr. f. exp. Path. u. Therap., 1915, XVII, 224; Howell, Jour. Infect. Dis., 1916, XIX, 63; Courmont and Devic, C. R. soc. biol. Paris, 1916, CLXIII, 534; Mendelson, Mil. Surg., 1916, XXXIX, 361; Weston, Jour. A. M. A., 1916, LXVI, 1089; Miller, Ill. Med. Jour., 1916, XXIX, 8; McCoy, Jour. A. M. A., 1917, LXVIII, 1401; Davison, Jour. Lab and Clin. Med., 1917, II, 607; Russell, Jour. A. M. A., 1919, LXXIII, 1863; Guerin-Valmale and Vayssiere, Gyn. et Obs., 1920, I, 217; Soper, Am. Jour. Pub. Health, 1920, X, 301; Grant, Jour. Am. Med. Assoc., 1921, LXXXVI, 514; Bunn, Ibid., 1159.

INDEX

- Abderhalden's sero-diagnosis of cancer, 770
 of dementia praecox, 771
 of pregnancy, 757
- Abortion, 425
- Abscess, blood in, 643
 indican in, 285
 of liver, sputum in, 36
 of lung, sputum in, 36
- Absorptive power of stomach, 93
- Acanthia lectularia, 177
- Acarus scabiei, 175
- Accidental albuminuria, 290
- Acetic acid in gastric contents, 71, 80 84
- Aceto-acetic acid, 207, 358
- Acetone bodies, 206, 350
 in blood, 534
 in urine, 206, 354
 determination of, 355
 significance of, 350
 tests for, 354
- Acetonemia, 534
- Acetonuria, 354
- Acholic stools, 115, 124
- Achorion Schönleini, 178
- Achrocythemia, 491
- Achromatophilia, 591
- Achroödextrin, 39, 89
- Achylia gastrica, 85, 96
- Acid, acetic, 71, 80, 84, 276
 aceto-acetic, 207, 358
 alloxypoteic, 225, 275
 amino, 89, 206, 272
 bile, 67, 116, 365
 butyric, 71, 80, 83, 276
 cholalic, 125, 206
 chondroitin-sulphuric, 206, 225, 281
 diacetic, 207, 358
 diamino, 90, 99, 272
 fatty, 13, 123, 131, 206, 276, 529
 formic, 276
 glycocholic, 125, 207, 365
 glycosuric, 368
 glycuronic, 206, 347
 hippuric, 207, 275, 380
 homogentisic, 207, 367
 hydrochloric, 54, 72
 hydroquinone-acetic, 367
 lactic, 71, 80, 81, 276
 nucleinic, 258, 280
 oxalic, 206, 277
 oxaluric, 206, 277
 β -oxybutyric, 207, 359
 oxymandelic, 207
 oxyproteic, 207, 225, 275
 phosphoric, 206, 214
 picric, 269, 302
 propionic, 276
 rosacic, 282
 rosolic, 22
 sulphuric, 223
- Acid, taurocholic, 125, 207, 365
 uric, 206, 255, 374, 508
 uroferic, 225
 uroleucic, 207, 367
- Acidemia, 456
- Acid-fast organisms, 21
- Acidity of gastric juice, 71
 of urine, 198, 200
- Acidophilic cells, 591
 granules, 591, 598
- Acidosis, 199, 351, 440
- Acid stains, 571
 unit, 202
- Actinomyces in sputum, 30
- Actinomycosis, 30
- Acute anterior poliomyelitis, 817
 bronchitis, sputum in, 34
 gastritis, gastric juice in, 97
 hemorrhage, anemia due to, 632
 infectious conjunctivitis, 52
 diseases, blood in, 646
 leukemia, 640
 nephritis, 193, 195, 205, 209, 216, 234,
 290, 294
 rheumatism, blood in, 654
 yellow atrophy of the liver, urine in,
 234, 272, 378
- Addison's disease, blood in, 646
- Adenin, 265
- Adler's test for blood, 119
- Adolescent albuminuria, 293
- Aerobic cultures, 845
 organisms, 845
- Æstivo-autumnal malaria, 664, 667
- Agar, ameba, 842
 ascitic, 843
 blood, 842
 dextrose, 842
 glycerin, 842
 hydrocele, 843
 litmus lactose, 842
 nutrient, 841
- Age, effect of, on red blood cells, 587
 on white blood cells, 618
- Agglutination, 701, 710
- Agglutinins, 701
- Agglutinophore, 701
- Agonal leucocytosis, 612
- Air in sputum, 4
- Albert's stain for diphtheria bacilli, 4
- Albumin, determination of, 295, 301
 in exudates, 792
 in feces, 131
 in milk, 830
 in sputum, 5
 in transudates, 792
 in urine, 289
 of blood, 492
 quotient, 304, 494
 removal of, 303

- Albumin, serum, 289
 significance of, in urine, 289
 tests for, 294
 Albuminuria, accidental, 290
 adolescent, 293
 after baths, 290
 alimentary, 290
 colliquative, 293
 constitutional, 291
 cyclic, 292
 false, 290
 febrile, 293
 functional, 290
 hematogenous, 294
 hypostatic, 293
 intermittent, 291
 lordotic, 292
 mixed, 310
 neurotic, 294
 of the new-born, 291
 of pregnancy, 291
 orthostatic, 292
 orthotic, 292
 physiologic, 289
 post-infectious, 293
 postural, 292
 renal, 294
 structural, 294
 thermolytic, 307
 toxic, 294
 traumatic, 293
 true, 289
 vesicular, 290
 with definite renal lesions, 294
 Albumon, 492
 Albumose, Bence-Jones, 305
 in blood, 494
 in feces, 131
 in gastric contents, 89
 in urine, 308
 Albumosuria, 308
 alimentary, 310
 digestive, 310
 enterogenous, 309
 febrile, 310
 hematogenous, 309
 hepatogenous, 309
 myelopathic, 306
 pyogenic, 309
 renal, 310
 significance of, 309
 tests for, 308
 Alcohol-fast organisms, 21
 Aleukemic myelosis, 641
 Alimentary albuminuria, 290
 albumosuria, 310
 chloruria, 299
 galactosuria, 338, 345
 glycosuria, 314
 levulosuria, 338
 lipuria, 381
 pentosuria, 341
 Alkalemia, 456
 Alkaline phosphates, 215
 reserve of blood, 455
 tide of urine, 201
 Alkalinity of blood, 437
 of urine, 202
 Alkalinuria, 216
 Alkalosis, 456
 Alkaptonuria, 367
 Allantoin, 206, 276
 Allergy, 703
 Alloxur bases, 131, 265, 376
 Alloxypoteic acid, 206, 225, 275
 Almén-Nylander's test for glucose, 324
 Almén's tannic acid solution, 829
 Aloin test for blood, 117, 784
 Altitude, effect of, on red cells, 588
 Alveolar epithelial cells in sputum, 111
 Ambard's coefficient, 504
 Amblyochromatic erythroblasts, 585
 Amboceptor paper, 735
 Amboceptors, 700, 728, 735
 Ameba agar, 842
 classification of, 144
 cysts of, 147
 coli in feces, 145
 in mouth, 46
 in sputum, 32
 in urine, 402
 pulmonalis, 32
 Amebic dysentery, 145
 pyorrhea, 46
 American hook-worm, 165
 Amino-acetic acid, 272
 Amino acids in blood, 521
 in urine, 206, 272
 α -aminoisobutyl-acetic acid, 378
 Ammonia in blood, 516
 in urine, 250
 Ammoniemia, 516
 Ammonium magnesium phosphate in feces, 128
 in sputum, 14
 in urine, 383
 urate calculi, 403
 sediment, 382
 Amniotic fluid, 425
 Amount of blood, 429
 of cerebrospinal fluid, 1812
 of feces, 112
 of gastric juice, 64
 of sputum, 2
 of urine, 190
 Amyloid kidney, globulin in urine of, 304
 urine in, 192, 195, 295, 304
 Amylopsin, 110
 Amylosis, 37
 Anachlorhydria, 77
 Anaerobic cultures, 845
 organisms, 845
 Anaphylaxis, 874
 Ancylostoma duodenale, 164
 Ancylostomiasis, 164
 Andradi's indicator, 141
 Anemia, 624
 aplastic, 631
 Biermer's, 627
 chlorotic, 625
 definition of, 624
 due to acute hemorrhage, 632
 to acute infections, 635
 to bad air, 634
 to blood poisons, 635
 to chronic disease, 644
 to chronic hemorrhage, 633
 to inanition, 633

- Anemia, to intestinal parasites, 634
 Ehrlich's, 631
 febrile, 635
 hemolytic, 635
 infantum pseudoleukemica, 630
 leukanemia, 630
 lymphatic, 630
 of the South, 165
 of the tropics, 588
 primary pernicious, 627
 progressive pernicious, 627
 secondary, 631
 simple primary, 624
 splenic, 629
 von Jaksch's, 630
 Anemic degeneration, 591
 Anesthesia, changes in urine after, 353
 effects of, on blood, 644
 Angioneurotic hematuria, 390
 Anguillula aceti in urine, 402
 intestinalis et stercoralis, 160
 Anhydremia, 432
 Animal gum in urine, 347
 parasites in blood, 658
 in ear, 51
 in feces, 142
 in gastric contents, 70
 in sputum, 32
 in urine, 402
 Ankylostomum duodenale, 164
 Anopheles crucians, 659
 bifurcatus, 659
 ludlowi, 659
 maculipennis, 659
 plumbeus, 659
 punctipennis, 659
 quadrifaculatus, 659
 Anterior urethritis, 388
 Anthracosis, 4, 37
 Antiambocceptors, 700
 Antibodies, 697, 720, 727
 Anticomplement, 700
 Antiformin method for tubercle bacilli, 18
 Antigens, 697, 721, 724, 730, 737, 744
 Antihemolysins, 700
 Antihuman amboceptor, 735
 Antimeningococcic serum, 816
 Antisheep amboceptor, 728
 Antitoxins, 697
 Antityphoid vaccination 717, 877
 Anuria, 193
 Aplastic anemia, 631
 Appearance of blood, 435
 of exudates, 792
 of feces, 113
 of gastric contents, 64
 of leucocytes, 597
 of milk, 827
 of red cells, 580
 of semen, 416
 of spinal fluid, 812
 of sputum, 4
 of transudates, 791
 of urine, 194
 Appetite juice, 55
 Arabinose, 341
 Arginin, 273
 Arneth's classification of neutrophils, 600
 Arnold's test for diacetic acid, 359
 Arnold-Volhard method for chlorids, 211
 Arterial blood, 436
 Arthropoda, 174
 Ascaridæ in feces, 158
 Ascaris alata, 159
 caniculæ, 159
 canis, 159
 canis et martis, 173
 cati, 159
 felis, 159
 græcorum, 159
 lumbricoides, 158
 lumbricus canis, 159
 marginata, 159
 mystax, 159
 teres, 159
 trichiura, 162
 tricuspidata, 159
 vermicularis, 159
 visceralis et renalis, 173
 wernerii, 159
 Ascitic agar, 843
 fluid, cytology of, 807
 Asexual cycle of malarial parasite, 662
 Ash-free diet of Taylor, 210
 Asiatic cholera, feces in, 138
 organism of, 138
 Aspergillus flavus, 17
 fumigatus, 17
 in aural secretion, 51
 in sputum, 17
 niger, 17
 subfuscus, 17
 Assimilation limit, 314
 Asthma, bronchial, 36
 eosinophilia in, 10, 36, 615
 fusiform bacillus in, 36
 sputum in, 36
 Aural secretion, 51
 larvæ in, 51
 molds in, 51
 Autotoxic enterogenous cyanosis, 590
 Autovaccines, 702, 876
 Avery's method of typing pneumococci, 27
 AzoöspERMATISM, 418
 Azotorrhea, 123
 Babcock's method for fat in milk, 831
 Bacillary dysentery, 141
 index, 702
 Bacilluria, 401
 Bacillus aerogenes capsulatus, 142, 858
 acidophilus, 138
 anthracis, 30
 bißidus, 138
 botulinus, 863
 coli communis, 51, 138, 401, 691, 852
 comma, 68, 138, 864
 Ducrey's, 798
 euteritidis sporogenes, 859
 fallax, 861
 histolyticus, 861
 hodgkini, 641, 850
 icteroides, 681
 lepræ, 24
 mallei, 30
 mucosus capsulatus, 28, 51
 oedematicus, 861
 oedematis maligni, 860

- Bacillus of Boas-Oppler**, 60
 of Bordet-Gengou, 29, 851
 of bubonic plague, 29
 of diphtheria, 42, 848
 of dysentery, 141, 855
 of Finkler-Prior, 68, 139, 378
 of Friedländer, 28, 51
 of glanders, 30
 of Hansen, 24
 of Hoffman, 44, 849
 of influenza, 28, 850
 of Kitasato and Yersin, 29
 of Klebs-Löffler, 42, 848
 of Koch, 18, 142, 399, 695, 795
 of Koch-Weeks, 52
 of Morax-Axenfeld, 52
 of ozena, 50
 of Perez, 50
 of Pfeiffer, 28, 36, 850
 of Sanarelli, 681
 of Shiga, 141, 855
 of soft chancre, 798
 of tuberculosis, 18, 142, 399, 695, 795
 of Tunncliffe, 49
 of Vincent, 44
 of Welch, 142, 858
 of whooping-cough, 29, 851
 paratyphoid, 140, 690
 perfringens, 859
 pertussis, 29, 851
 pestis, 29
 pseudo-diphtheria, 44, 849
 pyocyaneus, 4
 rhinitis, 49
 smegma, 25, 399, 798
 sporogenes, 862
 tetani, 863
 timothy, 25
 typhosus, 29, 139, 401, 689, 852
 ulceris cancrisi, 798
 xerosis, 44
 X of Sternberg, 681
- Bacteria in blood**, 687
 in conjunctiva, 52
 in ear, 51
 in exudates, 795
 in feces, 136
 in gastric contents, 69
 in milk, 834
 in mouth, 39
 in nasal secretions, 49
 in sputum, 14
 in urine, 398
- Bacterial flora of feces**, 138
 of vagina, 422
 vaccines, 702, 873
- Bacteriemia**, 690
- Bacteriology of blood**, 687
 of cerebrospinal fluid, 815
 of exudates, 795
 of feces, 136
 of milk, 834
 of sputum, 14
 of urine, 398
- Bacteriolysins**, 699
- Bacteriolytic test of Pfeiffer**, 865
- Bacteriuria**, 401
- Balanitis, erosive and gangrenous**, 798
- Balantidium coli**, 151
- Baldwin's method for oxalic acid**, 278
- Bang's test for albumose**, 309
 for sugar, 329
- Banti's disease**, blood in, 629
- Barberio's test for semen**, 420
- Barber's itch**, organism of, 179
- Bases**, alloxur, 131, 206, 265, 376
 hexone, 90, 99, 273
 nuclein, 131, 206, 265, 376
 purin, 131, 206, 376
 xanthin, 131, 206, 265, 376
- Basic stains**, 571
- Basket cells**, 604
- Basophile leucocytes**, 602
- Basophiles**, 602
- Basophilia**, 593, 602
- Basophilic degeneration of red cells**, 593
 stippling of reds, 593
- Bass and Watkin's macroscopic agglutination test**, 715
- Baths**, albuminuria following, 290
 effect of, on red cells, 589
- Beckmann apparatus**, 464
- Bed bug**, 177
- Beef tape-worm**, 153
- Bell & Doisy's method for inorganic phosphates**, 221, 541
 for total phosphorus, 221, 542
 for organic phosphorus, 222
- Bence-Jones**, body, 305
 protein, 305
 amount of, 306
 significance of, 306
 tests for, 307
- Benedict and Murlin's test for amino acids in urine**, 273
- Benedict's test for sugar in blood**, 528
 in urine, 321
 for uric acid in blood, 508, 513
- Benzidin test for blood**, 119
- Benzoic acid**, 275
- Bial's test for pentose**, 343
- Biermer's anemia**, 627
- Bile acids in blood**, 534
 in feces, 125
 in gastric contents, 67
 in urine, 206, 365
 media, 853
 obtaining of, 109
 pigments in blood, 534
 in exudates, 792
 in feces, 116, 125
 in gastric contents, 65, 67
 in sputum, 3
 in urine, 206, 363
 significance of, 363
 tests for, 364
- Bilharzia hematobia**, 33, 687
- Bilharziasis**, 687
- Bilicyanin**, 125
- Bilifuscin**, 125, 363
- Bilihumin**, 125
- Biliprasin**, 115, 363
- Bilirubin**, 65, 116, 125, 363, 380, 534
- Biliverdin**, 65, 116, 125, 363
- Biologic test for blood**, 718
- Bismuth oxid in stools**, 115
 test for glucose, 324

- Biuret test for protein, 308
 Black's method for β -oxybutyric acid, 360
 Black sputum, 4
 urine, 197
 -water fever, 311
 Bladder, inflammation of, 202, 387
 tuberculosis of, 399
 Blastomycetes in skin, 182
 in sputum, 17
 Blastomycosis, 17, 182
 Blennorrhea, 422
 Blood, acetone in, 534
 after anesthesia, 644
 after splenectomy, 658
 after surgical intervention, 643
 agar, 842
 albumin in, 492
 alkalinity of, 437
 amino acids, in, 521
 ammonia in, 516
 bacteriology of, 687
 biliary constituents in, 534
 carbohydrates in, 523
 casts, 394
 cells, 548
 in exudates, 793
 in feces, 128
 in gastric contents, 67, 90
 in sputum, 3, 5, 11
 in urine, 362, 389
 chemical properties of, 466
 tests for, 117, 784
 cholesterin in, 530
 coagulation of, 460, 774
 color-index of, 491
 color of, 436
 constituents of, 466
 counting of cells of, 548, 554, 605
 creatinin in, 518
 crises, 585
 cryoscopy of, 464
 cultures, 687
 dust, 621
 electric conductivity of, 466
 enumeration of cells of, 548, 554, 605
 fat in, 529
 ferments of, 548
 fixation of smears of, 567
 formation of, 428
 fresh, 563
 gases in, 547
 glucose in, 523
 grouping of, 779
 hemoglobin of, 469
 in abscess formation, 643
 in acute infections, 646
 rheumatism, 654
 in Addison's disease, 646
 in aplastic anemia, 631
 in bilharziasis, 687
 in carcinoma, 656
 in chlorosis, 625
 in chronic diseases, 644
 tuberculosis, 644
 in diabetes mellitus, 644
 in diphtheria, 653
 in distomiasis, 687
 in filariasis, 674
 in gout, 645
 in kala-azar, 674
 in leprosy, 656
 in leukanemia, 630
 in leukemia, 636
 in malaria, 659
 in measles, 651
 in myxedema, 646
 inorganic constituents of, 535
 in pernicious anemia, 627
 in pertussis, 653
 in pneumonia, 647
 in primary anemia, 624
 in pseudoleukemia, 641
 in relapsing fever, 671
 in rickets, 646
 in Rocky Mountain spotted fever, 686
 in scarlet fever, 650
 in secondary anemia, 631
 in sleeping sickness, 672
 in splenic anemia, 629
 in syphilis, 655, 676
 in typhoid fever, 649, 689
 in varicella, 653
 in variola, 652
 in whooping-cough, 653
 in yellow fever, 681
 limitations of examinations of, 788
 medico-legal aspects of, 783
 tests for, 783
 morphology of, 563
 needle, 435
 nitrogen of, 495
 non-protein nitrogen of, 495
 obtaining of, 434
 odor of, 437
 osmotic pressure of, 464
 parasitology of, 658
 pathology of, 624
 general, 643
 special, 624
 physiology of, 428
 pigments of, 469
 plates, 562, 619
 appearance of, 619
 counting of, 562
 function of, 621
 number of, 620
 size of, 620
 staining properties of, 621
 poisons, 635
 properties of, 435, 466
 proteins of, 492
 reaction of, 437
 red cells of, 580
 serum reactions of, 710
 smears of, 565
 fixation of, 567
 preparation of, 565
 staining of, 570
 solids of, 468
 specific gravity of, 457
 spectroscopic tests for, 470, 787
 staining of smears of, 570
 tests for, 119, 784
 total solids of, 468
 urea in, 501
 uric acid in, 508
 value of examinations of, 788

- Blood, viscosity of, 450
 vital staining of, 579
 volume of, 429
 relations of elements of, 432
 white cells of, 597
- Bloody sputum, 3, 5, 11
- Bloor and Knudson's method for cholesterol esters, 533
- Bloor's method for cholesterolin, 532
- Boas' method for lactic acid, 83
 for estimating gastric motility, 92
 test for free hydrochloric acid, 73
 test-meal, 61
- Boas-Oppler bacillus, 69
- Body louse, 176
- Boggs' coagulometer, 462
 method for protein in milk, 829
- Bone-marrow, 428, 622
 function of, 428
 morphology of, 621
- Borax as preservative in milk, 834
- Bordet's potato medium, 851
- Boric acid as preservative, 834
- Bothriocephaloidea in feces, 156
bothriocephalus latissimus, 156
 latus, 156
 sp. Ijima et Kurimoto, 157
dibothriocephalus cordatus, 157
- Böttcher's crystals, 14, 416
- Bouillon, 839
- Bradshaw's myelopathic albumosuria, 306
- Breakfast, test, 60
- Bremer's blood-test in diabetes, 644
- Brem's method for grouping blood, 781
- Bricklayers' anemia, 166
- Brilliant green in culture media, 140
- Brine flotation loop method, 140
- Brodie-Russell coagulometer, 462
- Bronchial asthma, 36
 spirochetosis, 37
 stones, 8
- Bronchioliths, 8
 exudativa, 7
- Bronchitis, acute, 34
 chronic, 35
 eosinophilic, 10
 fetid, 35
 fibrinous, 35
 putrid, 35
- Broncho-pneumonia, sputum of, 34
- Bronfenbrenner's modification of Abderhalden test, 767
- Bruck's test, 772
- Bunge-Trantenroth's method for tubercle bacilli, 25
- Burri's method for spirochæte, 800
- Busk's intestinal fluke, 171
- Butyric acid in gastric contents, 71, 80, 83
- Cabot's ring bodies, 594
- Cachexial fever, 674
- Cadaverin, 131, 377
- Caffein, 266
- Calcium carbonate calculi, 405
 sediment, 384
 of blood, 544
 of urine, 229
 oxalate calculi, 403
- Calcium oxalate crystals in sputum, 14
 in urine, 376
 phosphate calculi, 405
 sediment, 381
 soaps in feces, 128
 sulphate sediment, 380
- Calculi, ammonium urate, 403
 biliary, 126
 bronchial, 8
 calcium carbonate, 405
 oxalate, 403
 classification of, 402
 cystin, 405
 examination of, 404
 formation of, 402
 hepatic, 126
 intestinal, 126
 nasal, 50
 phosphatic, 405
 pulmonary, 9
 renal, 402
 table for examination of, 404
 ureteral, 402
 urethral, 402
 uric acid, 403
 urostealith, 406
 vesical, 402
 xanthin, 405
- Calmette's tuberculin reaction, 705
- Cambridge's reaction, 344
- Cancer (see Carcinoma)
- Cane sugar, absorption of, 132
 digestion of, 71
 in urine, 347
- Capsule, staining of, 693
- Carbohydrates, digestion of, 39, 71, 89, 125, 132
 in blood, 523
 in exudates, 792
 in feces, 125, 132
 in milk, 832
 in urine, 313
- Carbol-fuchsin solution, 21
- Carbonates in urine, 228
- Carbon dioxide capacity of blood, Van Slyke and Cullen's Method for, 441
 hemoglobin, 474
 in blood, 474
 monoxid hemoglobin, 473
 poisoning, 473
- Carcinoma, Abderhalden's test in, 770
 blood in, 656
 cells in exudates, 806
 fragments in feces, 127
 in gastric contents, 70, 100
 in urine, 398
 of cervix, 426
 of kidney, 389, 398
 of pleura, 806
 of rectum, 127
 of stomach, 98
 of uterus, 426
- Cardiac albuminuria, 291
 edema, 191
 pleurisy, 807
- Carnin, 265
- Casein in feces, 123
 appearance of, 123

- Casein in feces, Leiner's test for, 123
 in milk, 830
 Castellani's disease, 37
 spirochete, 37
 Casts, 391
 blood, 394
 chemistry of, 391
 colloid, 395
 epithelial, 394
 fatty, 394
 fibrinous, 394
 in sputum, 8
 granular, 392
 hyaline, 391
 mixed, 392
 origin of, 391
 prostatic, 418
 pseudo, 396
 pus, 394
 significance of, 392, 396
 size of, 391
 staining of, 392
 testicular, 418
 true, 391
 waxy, 393
 Catalase, 619
 Cataphylaxis, 859
 Catarrhal stomatitis, 41
 Cellulose in feces, 125
 Centrifugation, 372
 Cercomonads in feces, 150
 in sputum, 32
 in urine, 402
 in vaginal secretions, 423
 Cercomonas coli hominis, 149
 hominis, 150
 intestinalis, 150
 seu Bodo urinary, 149
 Cerebrospinal fluid, 810
 bacteriology of, 814
 chemistry of, 813
 cytology of, 814
 in nasal secretion, 50
 microscopy of, 814
 obtaining of, 811
 pressure of, 811
 properties of, 812
 syphilis, 818
 Cerumen, 51
 Cestodes in feces, 151
 in sputum, 32
 Chalicosis, 4, 37
 Chancre, organism of, 798
 Chancroid, organism of, 798
 Character of blood, 435
 of exudates, 792
 of feces, 113
 of sputum, 4
 of urine, 194
 Charcoal in feces, 107
 Charcot-Leyden crystals in feces, 129
 in sputum, 14, 33, 36
 Cheesy masses in sputum, 7
 Chemical fixation of smears, 569
 Chemotaxis, 701
 Childhood, red cells in, 595
 white cells in, 618
 Chinese liver-fluke, 172
 Chinovose, 341
 Chloremia, 491
 Chlorid excretion in urine, 208, 408
 retention, 209, 536
 Chlorids of the blood, 535
 of the plasma, 535
 of the urine, 208
 amount of, 208
 estimation of, 211
 variations of, 209
 Chloromata, 4
 Chlorosis, 625
 Chlorotic anemia, 625
 Chloruria, 208, 408
 Cholecyanin in feces, 115
 in urine, 363
 Cholelithiasis, 126, 530
 Cholemia, 534
 Cholera red reaction, 865
 spirillum, characteristics of, 864
 in feces, 138
 Cholesterol in blood, 530
 crystals in feces, 129
 in sputum, 13
 in urine, 381
 Choletelin in urine, 363
 Choluria, 363
 Chondroitin-sulphuric acid, 206, 225, 281
 Chromogenic bacteria in sputum, 4
 Chromogens in urine, 281
 Chronic bronchitis, sputum in, 35
 diseases, blood in, 644, 655
 gastritis, gastric juice in, 97
 nephritis, urine in, 193, 195, 205, 209,
 216, 295
 Chyloid exudates, 793
 Chylous exudates, 793
 Chyluria, 196, 381
 Chymosin, 70, 88
 Cimænomonas hominis, 149
 Cimex lectularius, 177
 Cladocœlium hepaticum, 170
 Clark's method for elastic tissue, 12
 Clay-colored stools, 115, 125
 Cleaning glass-ware, 563
 Clearing of urine, 295, 333
 Clonorchis sinensis, 172
 Coagulation of blood, 460, 774
 of exudates, 792
 of milk, 828
 of urine, 301
 time of blood, 460
 Coagulometer of Boggis, 462
 of Dorrance, 461
 of Rudolf, 462
 of Russell-Brodie, 462
 Coagulo reaction for syphilis, 773
 Coal pigment in sputum, 4, 37
 Coarsely granular cells of Schultze, 601
 Coating of the tongue, 41
 Coccidioidal granuloma, 183
 Coccidioides immitis, 183
 Coccidium hominis, 149
 perforans, 149
 Coccobacillus foetidus ozenæ, 50
 Coefficient, creatinin, 267
 of Ambard, 504
 of Haeser, 204
 of Haines, 204
 of Long, 204

- Coefficient, refraction, 493
 Colitis, catarrhal, 124
 malignant, 127
 mucous, 120
 Collection of feces, 106
 of gastric contents, 56
 of puncture fluids, 791
 of sputum, 2
 of urine, 189
 Collignon and Pilod's test, 816
 Colloidal benzoïn test, 823
 gold test, 821
 Colloid casts, 391
 cysts, 808
 Colloidal nitrogen, 275
 Colon bacillus, 51, 138, 401, 691, 852
 Color-index of blood cells, 491
 of blood, 491
 of exudates, 792
 of feces, 114
 of gastric contents, 65
 of sputum, 3
 of urine, 195
 Colorimeter, 270
 Colorimetric method for H ion concentra-
 tion, 448
 Colostrum, 825
 Coma diabeticum, 353
 Combined hydrochloric acid, 78
 Comma bacillus of Koch, 68, 138, 864
 Common flea, 178
 liver-fluke, 170
 Complement, 700, 729, 735
 fixation test, 721, 756
 for gonorrhœa, 756
 for syphilis, 721
 for tuberculosis, 756
 preservation of, 729
 Complementophile, 700
 Composition of blood, 466
 of milk, 826
 of urine, 206
 Concretions, biliary, 126
 bronchioliths, 8
 coproliths, 127
 enteroliths, 126
 intestinal, 126
 in sputum, 8
 nasal, 50
 pneumoliths, 9
 renal, 402
 vesical, 402
 Conductivity, electric, of blood, 466
 of urine, 408
 Congo-red test, 72
 Conjugated glycuronic acids, 348
 Conjunctival secretions, 51
 Conjunctivitis, diphtheritic, 52
 gonorrheal, 52
 infectious, 52
 venal, 53
 Consistency of blood, 459
 of feces, 113
 of gastric contents, 66
 of milk, 827
 of spinal fluid, 812
 of sputum, 2
 of urine, 194
 Constipation, 113
 Coproliths, 127
 Corpora amylacea, 418
 Corynebacterium granulomatis maligni,
 850
 Cough, whooping, blood in, 653
 organism of, 29, 851
 Counting of blood plates, 562
 of pus cells, 389
 of red cells, 548
 of white cells, 559
 Crab louse, 176
 Creatin in blood, 521
 in urine, 266, 271
 Creatinin coefficient, 267
 estimation of, 269, 520
 metabolism of, 266, 518
 tests for, 268
 variations of, 266, 518
 Creatorrhœa, 123
 Crenation, 581
 Crescents in blood, 667
 Crises, blood, 585
 Cryoscopy of blood, 464
 of urine, 407
 Crystals in feces, 128
 in gastric contents, 70
 in semen, 416
 in sputum, 13
 in urine, 374
 Csoska's method for acetone, 355
 Culex mosquito, 681
 Cultures, aerobic, 845
 anaerobic, 845
 blood, 687
 brilliant green in, 140
 media, 839
 throat, 42
 urine, 398
 Cunningham's stain, 576
 Curds in feces, 123
 Curschmann's spirals, 7, 33, 36
 Cutaneous reactions, 703
 Cyclic albuminuria, 292
 Cylindroids, 395
 Cylindruria, 396
 Cyst, colloid, 808
 dermoid, 809
 fluids, 807
 hydatid, 810
 hydrocele, 809
 hydronephrotic, 809
 myxoid, 808
 ovarian, 807
 pancreatic, 810
 papillary, 809
 parovarian, 809
 serous, 807
 spermatocele, 809
 Cystein, 225
 Cysticercus cellulosæ, 153
 Cystin, 225
 calculi, 405
 sediment, 377
 Cystinuria, 377
 Cystitis, 202, 387, 401
 Cystospermium hominis, 149
 Cystotænia solium, 153
 Cytology in cardiac pleurisy, 807
 in malignant pleurisy, 806

- Cytology in nephritic pleurisy, 807
 in pneumococcus pleurisy, 806
 in primary tubercular pleurisy, 805
 in secondary tubercular pleurisy, 806
 in streptococcus pleurisy, 806
 in typhoid pleurisy, 806
 of ascitic fluid, 807
 of cerebrospinal fluid, 814
 of exudates, 803
 of peritoneum, 807
 of pleura, 805
 of normal-fluids, 804
 of sputum, 10
 technic of, 803
 Cytophile, 697
 Dahlia stain, 602
 Daland's hematocrit, 432
 Dare's hemoglobinometer, 485
 Darling's method of staining amebæ, 147
 Day urine, 191
 Dechloridization, 210
 Defense rupture, 859
 Deficit of hydrochloric acid, 80
 Definitive host, 153
 Degenerated forms of red cells, 592
 of white cells, 604
 Degeneration, anemic, 591
 hemoglobinemic, 594
 Degree of tolerance, 314
 Delayed chloroform poisoning, 353
 Wassermann reaction, 738
 Dementia præcox, Abderhalden's test in,
 771
 Demodex folliculorum, 175
 Deniges' reaction for cystin, 405
 Dermacentor Andersoni, 686
 venustus, 686
 Dermacentroxenus rickettsi, 687
 Dermoid cysts, 809
 Desmoid bag, 94
 Deutero-albumose, 89, 308
 Dextrin in urine, 347
 Dextrose agar, 842
 Diabetes alternans, 258
 insipidus, 192
 mellitus, 192, 195, 315, 351
 blood in, 644
 Bremer's test in, 644
 lipemia in, 644
 urine in, 192, 195, 315, 351
 Williamson's test in, 645
 phosphatic, 217
 renal, 314
 Diabetic coma, 351, 353
 Diacanthos polycephalus, 166
 Diacetic acid in urine, 358
 Diagnosis, functional, 406
 Diagnostic value of agglutination test,
 716
 of complement fixation test, 750
 of precipitin test, 720
 Diamines in feces, 131
 in urine, 377
 Diamino acids, 90, 99, 273
 Diaminuria, 377
 Diarrhea, 113
 Diastase in urine, 279
 Diazo reaction, 368
 Dibothriocephalus cordatus, 157
 latus, 156
 Dibothrium latum, 156
 Diet of Folin, 107
 of Schmidt and Strasburger, 106
 of Taylor, 210
 Dieudonne's medium, 865
 Differential counting, 605
 Diffusible alkalinity of blood, 437
 Digestion, gastric, 89
 intestinal, 111, 129
 leucocytosis of, 608
 products of, 89, 111
 Digestive insufficiency, 121
 Dilatation of stomach, 90, 91
 Diluting fluids for blood, 553, 559
 Dimethylaminoazobenzol test, 73
 Dimethylaminobenzaldehyd reaction, 371
 Dimorphous muris, 150
 Diphtheria, bacillus of, 42, 848
 blood in, 653
 carriers, 43
 taking smear in, 42
 Diphtheritic conjunctivitis, 52
 laryngitis 42
 Diphylobothrium latum, 156
 Diplacanthus nana, 154
 Diplococcus cinereus, 869
 crassus, 869, 872
 flavus, 869, 872
 intracellularis meningitidis, 815, 871
 lanceolatus, 26, 34
 of Bonome, 815
 of Fraenkel, 26, 34, 692, 865
 of Jaeger and Heubner, 815
 of Neisser, 795, 869
 of Weichselbaum, 815, 871
 pneumoniæ, 26, 34, 692, 865
 reniformis, 797
 Diplogonoporus grandis, 157
 Diplomellituria, 318
 Dipylidium caninum, 154
 cucumerinum, 154
 Distoma capense, 687
 pulmonale, 32
 Ringeri, 32
 Westermanii, 32
 Distomiasis, 687
 Distomum buski, 171
 caviæ, 170
 conus, 172
 crassum, 171
 hematobium, 33, 687
 hepaticum, 170
 hepatis endemicum seu perniciosum,
 172
 innocuum, 172
 japonicum, 172
 lanceolatum, 172
 sibiricum, 172
 sinense, 172
 spathulatum, 172
 tenuicolle, 172
 Ditrachyceros rudis, 166
 Dittrich's plugs, 7
 Doehmius ancylostomum, 164
 duodenalis, 164
 Dock's test meal, 61
 Dohle's inclusion bodies, 651

- Donaldson's method for staining amebic cysts, 147
- Donné's test for pus, 389
- Donogany's test for hemoglobin, 312
- Doremus ureometer, 243
- Dorrance's method for coagulation-time, 461
- Drigalski and Conradi's media, 139
- Drop method for Wassermann test, 733
- Dropsical cells, 586, 625
- Dropsy of chorionic villi, 425
- Drugs, effects of, on blood, 491, 589
reactions of, in urine, 196
- Dry test of feces for blood, 119
- Ducrey's bacillus, 798
- Durham's hemocytometer, 561
- Dum-dum fever, 674
- Dwarf tape-worm, 154
- Dysentery, amebic, 145
bacillary, 141
- Dysmenorrhea, 424
- Dyspepsia, 98
- Earthy phosphates, 214, 216
- Eberth's bacillus, 29, 139, 401, 689, 852
- Echinococcus in feces, 155
in sputum, 9
in urine, 402
- Ectasis gastric, 92
- Eel, vinegar, 402
- Effusions, pleuritic, 805
- Egg-yellow reaction, 369
- Egyptian chlorosis, 166
- Ehrlich's anemia, 631
anemic degeneration, 591
classification of leucocytes, 597
dahlia stain, 602
diazo reaction, 368
dimethylaminobenzaldehyd reaction, 371
egg-yellow reaction, 369
hemoglobinemic degeneration, 592
side-chain theory, 697
tri-acid stain, 574
triple stain, 574
- Einhorn's method for total acidity, 79
- saccharometer, 336
- Elastic tissue in feces, 123
in sputum, 12
- Electric conductivity of blood, 466
of urine, 408
- Empyema, perforating, 37
- Endameba buccalis, 46
coli, 149
gingivalis, 46
histolytica, 145
tetragena, 146
- Endo medium, 141
- Endotheliosis, 806
- Enteritis, catarrhal, 118
malignant, 127
membranous, 120
mucous, 120
- Enterokinase, 110
- Enteroliths, 126
- Enthelmintha, 151
- Entozoa in feces, 151
- Enumeration of blood cells, 548, 559, 562, 605
of pus cells, 389
- Eosin-hematoxylin stain, 573
methylene-blue stain, 575
- Eosinophiles, 601
- Eosinophilia, 615
- Eosinophilic bronchitis, 10
- Epicritic elimination of nitrogen, 234
polyuria, 192
- Epidemic cerebrospinal meningitis, 815
- Epiguanin, 265
- Episarkin, 265
- Epistaxis, Gull's renal, 390
- Epithelial casts, 394
cells in feces, 128
in gastric contents, 68
in semen, 418
in sputum, 11
in urine, 384
- Erepsin, 110
- Erosive balanitis, 798
- Error in cell counting, 560
- Erythrasma, 182
- Erythroblasts, 584
- Erythrocytes, 580
appearance of, 580
color-index of, 491
counting of, 548
crenation of, 581
degenerations of, 592
formation of, 428
functions of, 596
isotonicity of, 594
nucleation of, 584
number of, 587
pathological types of, 582, 592
recognition of, in stains, 783
resistance of, 594
rouleaux formation of, 581
shape of, 582
size of, 582
staining properties of, 591
structure of, 580
variations of, 587
- Erythrocytometer, 550
- Erythrocytosis, 590
- Erythrodextrin, 39, 89
- Esbach's method for albumin, 302
- Essential albuminuria, 294
pentosuria, 341
renal hematuria, 390
- Esterification method of Fischer, 273
- Estivo-autumnal malaria, 664, 667
- Ethereal sulphates, 224, 227
- Euchlorhydria, 77
- Euglobulin, 304
- European cat-fluke, 172
hook-worm, 164
- Eustrongylus gigas, 173
visceralis, 173
- Ewald test-meal, 61
and Siever's method for gastric motility, 92
- Exercise, effect of, on red cells, 588
leucocytosis due to, 613
- Extraction method for fat, 832
- Extraneous material in sputum, 9
- Extruded intracellulars, 662
- Exudates, 790
bacteriology of, 795
chyloid, 793

- Exudates, chylous, 793**
 conjunctival, 52
 cytology of, 803
 formation of, 790
 hemorrhagic, 793
 obtaining of, 791
 peritoneal, 807
 pleural, 805
 properties of, 792
 purulent, 794
 putrid, 794
 serofibrinous, 792
 serous, 792
 urethral, 795
- False albuminuria, 290**
Famine fever, 671
Fasciola hepatica, 170
 humana, 170
Fasciolopsis buski, 171
Fasting stomach, contents of, 66
Fat in blood, 529
 in exudates, 792
 in feces, 124, 131
 in milk, 831
 in urine, 381
Fatty acids in blood, 529
 in exudates, 792
 in feces, 124, 131
 in sputum, 13
 in urine, 276
 casts, 394
 granules in leucocytes, 599
 stools, 115, 124
Favus, 178
Febrile albuminuria, 293
 albumosuria, 310
 anemia, 635
 diseases, blood in, 646
 urine, 195
Fecal vomitus, 68
Feces, 105
 amount of, 112
 bacteriology of, 136
 bile acids in, 125
 biliary pigments in, 116, 125
 blood in, 116
 carbohydrates in, 125, 132
 chemical examination of, 129
 color of, 114
 concretions in, 126
 consistency of, 113
 crystals in, 128
 fat in, 124, 131
 food remnants in, 121
 formed, 113
 macroscopic examination of, 111
 marking of, 107
 microscopic examination of, 127
 morphological elements in, 128
 mucus in, 119
 normal, 105
 odor of, 114
 parasitology of, 142
 protein in, 123
 pus in, 121
 reaction of, 129
 tissue fragments in, 127
 total nitrogen of, 130
 Feces, total solids of, 130
 unformed, 113
Fehling's test for glucose, qualitative, 322
Female secretions, 421
Fermentation method of Schmidt, 133
 test for diphtheria bacillus, 44
 for glucose, 325, 336
Fermentative dyspepsia, 125
Ferments in blood, 548
 in feces, 110
 in gastric juice, 71, 84
 in leucocytes, 548
 in sputum, 6
 intestinal, 110
 in urine, 279
 pancreatic, 110
Ferrocyanide test for albumin, 300
Ferrometer of Jolles, 546
Fibers, elastic, 12, 123
 muscle, in feces, 123
 in gastric contents, 67
Fibrin ferment, 460
 in blood, 463
 in urine, 312
 network, 463
 significance of, 463
 tests for, 313
Fibrinogen, 460
Fibrinous casts in sputum, 8
 in urine, 394
Fibrinuria, 312
Filaria Bancrofti, 674
 in blood, 674
 in urine, 381
 nocturna, 674
 sanguinis hominis, 674
Filariasis, 674
Finely granular cells of Schultze, 599
Fischer's esterification method, 273
 test-meal, 62
Fish tape-worm, 156
Fittipaldi's method for albumose, 308
Fixation of complement, 721.
 of smears, by chemicals, 569
 by heat, 567
Fixed alkalinity, 202
Flagella, staining of, 847
Flagellata in feces, 149
 in sputum, 32
 in urine, 402
Flat worms, 151
Fleischl-Miescher hemometer, 482
Flexner's serum, 816
Florence's test for seminal fluid, 419
Fluids, diluent for blood, 553, 559
Fluke-worms, 157, 169
Folin and Bell's direct nesslerization
 method for ammonia in urine, 254
Folin and Denis' direct nesslerization
 method for total N in urine, 238
 method for lactose in milk, 832
Folin and Macallum's method for ammonia
 in urine, 253
Folin and McEllroy's test for sugar in
 urine, 321
Folin and Peck's quantitative test for
 sugar in urine, 328
Folin and Wright's simplified Kjeldahl
 method, 237

- Folin and Wu's method for creatin in
 blood, 521
 creatinin in blood, 520
 non-protein N in blood, 496
 sugar in blood, 526
 urea in blood, 501
 uric acid in blood, 511
 in urine, 263
- Folin and Youngburg's direct nesslerization
 method for urea in urine, 249
- Folin's method for acetone, 357
 for acidity of urine, 199
 for amino acids in blood, 522
 in urine, 274
 for ammonia, 253
 for creatin, 271
 for creatinin, 269, 271
 for free mineral acidity, 200
 for indican, 288
 for sulphates, 226
 for urea, 245
 for uric acid, 259
 standard diet, 107
- Fontana's stain, 800
- Foreign bodies in sputum, 9
- Form of stools, 113
- Formaldehyd as preservative, 190, 834
- Formalin method for ammonia, 255
- Formation of blood, 428
 of casts, 391
 of exudates, 790
- Fourth venereal disease, 798
- Fractional examination of gastric juice, 58
- Fraenkel's diplococcus, 26, 34, 692, 865
- Fragments of tissue in feces, 127
 in gastric contents, 70
 in sputum, 12
 in urine, 398
- Free hydrochloric acid, 72
 amount of, 75
 detection of, 73
 determination of, 74
 formation of, 55
 significance of, 76
 variations of, 77
- Freezing point of blood, 464
 of urine, 407
- Fresh blood, 564
- Friedländer's bacillus, 28, 51
- Frommer's test for acetone, 355
- Fuchsin-aldehyd reaction for colon bacilli,
 854
- Fucose, 341
- Functional albuminuria, 290
 diagnosis, 406
 hematuria, 390
- Functions of gastric ferments, 84, 91
 of intestinal ferments, 110
 of leucocytes, 618
 of red cells, 596
- Fusaria mystax, 159
 vermicularis, 159
- Fusiform bacillus of Vincent, 44
- Futcher and Lazear's fixation method, 570
 malarial stain, 578
- Gabbet's staining method, 22
- Gabritschewsky's polychromatophilia, 592
- Gaffky's table, 23
- Galactosuria, 338, 345
- Gall stones in feces, 126
 appearance of, 126
 composition of, 126
- Gamete, 662
- Gametocyte, 662
- Gametochizonts, 662
- Gangrenous balanitis, 798
- Gas bacillus, 142, 858
- Gases in blood, 547
 in feces, 105, 134
 in gastric contents, 90
- Gastric carcinoma, 98
 contents, 54
 acetone in, 91
 after test meals, 68
 amino acids in, 90
 bacteria in, 69
 blood in, 66, 68, 90
 crystals in, 70
 digestion products in, 89
 epithelial cells in, 68
 food remnants in, 69
 fractional withdrawal of, 58
 from fasting stomach, 66
 from vomitus, 66
 gases in, 90
 indirect examination of, 94
 macroscopic examination of, 64
 microscopic examination of, 68
 mucus in, 66, 68, 98
 obtaining of, 56
 protozoa in, 70
 pus in, 68
 tissue fragments in, 70
- crises, 96
- juice, 64
 acetic acid in, 71, 80, 84
 acidity of, 71
 amount of, 64
 butyric acid in, 71, 80, 83
 combined hydrochloric acid in, 78
 composition of, 70
 deficit of hydrochloric acid in, 80
 ferments of, 70, 84
 free hydrochloric acid in, 72, 99
 hyperacidity of, 72, 78, 95
 hypersecretion of, 96
 hypoacidity of, 71, 77
 in disease, 95
 lactic acid in, 81, 99
 organic acids in, 80
 Pawlow's work on, 55
 properties of, 64
 secretion of, 55
 motility, 91
 ulcer, 98
- Gastrin, 55
- Gastritis, acute, 97
 atrophic, 97
 chronic, 97
- Gastrosuccorria, 96
- Gelatin, nutrient, 841
- Genital organs, secretions of, 416
- Genito-urinary tuberculosis, 387, 399
- Gerhardt's test for diacetic acid, 358
- Gettler and Jackson's method of preparing
 colloidal gold solution, 821
- Ghoreyeb's stain for spirochæte, 799

- Giardia intestinalis*, 150
 Giemsa's stain, 577, 799
 Gigantoblasts, 586
 Giantocytes, 583
 Glanders, bacillus of, 30
 Globular decolorization, 592
 Globulin-albumin ratio, 304
 Globulin in blood, 304, 492
 in exudates, 792
 in milk, 830
 in urine, 304
 significance of, 304
 tests for, 304
 Glomerular insufficiency, 414
 Glossina palpalis, 672
 Glucose agar, 742
 in the blood, 523
 in the urine, 313
 determination of, 328
 significance of, 313
 tests for, 318
 Glutoid capsules, 111
 Glycemia, 523
 Glycerin agar, 742
 Glycocholic acid, 125, 206, 365
 Glycocol, 273
 Glycogen in the blood, 529
 Glycosuria, 314
 alimentary, 314
 after poisoning, 318
 after use of drugs, 318
 diabetic, 316
 e saccharo, 314
 ex amylo, 314
 masked, 325
 neuro-hepatogenous, 317
 occult, 325
 physiologic, 313
 salt, 313
 transitory, 314
 Glycosuric acid, 368
 Glycuronic acid, 347
 Glycyl-tryptophan test, 101
 Gmelin's reaction for biliary pigments, 364
 Goldhorn's stain, 679
 Goldschmidt's test for glycuronic acid, 350
 Gonococcus, 795, 869
 Gonorrhea, complement-fixation test in, 756
 Gonorrheal conjunctivitis, 52
 stomatitis, 45
 threads, 388, 395, 797
 urethritis, 797
 Goodman and Stern's method for albumin, 303
 Gout, blood in, 515, 645
 perinuclear granules in, 600
 urine in, 258
 Gowers' hemoglobinometer, 486
 Gram-negative organisms, 796
 Gram-positive organisms, 796
 Gram's stain, 796
 Granular casts, 392
 cells in blood, 599, 601, 602
 in prostatic fluid, 417
 in sputum, 10, 11
 degeneration, 591
 Granules in blood, acidophile, 601
 basophile, 593, 602
 of Grawitz, 593
 Granules in blood, Ehrlich's α , 601
 β , 601
 γ , 602
 δ , 602
 ϵ , 599
 eosinophile, 601
 fatty, 604
 glycogen, 605
 Grawitz, 593
 hemoconien, 621
 in malaria, 662, 663, 665
 mast cell, 602
 melanin, 662, 663, 665
 Neusser's, 600
 neutrophile, 599
 oxyphilic, 601
 perinuclear, 600
 sudanophile, 604
 in sputum, 11
 Grape-sugar in urine, 313
 Gravel in urine, 402
 Grawitz' basophilia, 593
 Green sputum, 3
 vomitus, 67
 Griess-Ilosvay reagent, 39
 Grinders' rot, 4
 Gross' method for trypsin, 110
 Ground itch, 165
 Grouping of blood, 779
 Gruber-Widal reaction, 711
 Guaic test for blood, 117, 784
 Guanin, 265
 Guillain Laroche and Lechelle's colloidal
 benzoin test, 823
 Gull's renal-epistaxis, 390
 Gum, animal, in urine, 347
 Gummatous lymphoma, 643
 Gunning's mixture, 235
 test for acetone, 355
 Günzburg's package, 94
 reagent, 73
 test for free hydrochloric acid, 73
 Gynecophorus hæmatobius, 402, 687
 Haeser's coefficient, 204
 Haines' coefficient, 204
 test for glucose, qualitative, 322
 quantitative, 332
 Haldane and Smith's method for volume of
 blood, 430
 Halitus sanguinis, 437
 Hammarsten's test for biliary pigments, 365
 Hammerschlag's method for specific
 gravity, 458
 for pepsin, 86
 Haptines, 697
 Haptophore, 697
 Hard chancre, organism of, 798
 Hart's method for β -oxybutyric acid, 360
 Harvest bug, 175
 Hayem's solution, 553
 Hay fever, 50
 Hay's test for bile acids, 366
 Head louse, 176
 Heart disease cells, 11, 35
 pleurisy of, 807
 Heat fixation of smears, 567
 test for albumin, 296
 Hehner-Maly method for organic acids, 80

- Hektoen's precipitin test for semen, 420
 Heller's table for examination of calculi, 404
 test for albumin, 297
 for hemoglobin, 312
 Hemameba malariae, 663
 vivax, 660
 Hemamebiasis, 659
 Hematemesis, 3, 67
 Hematin, 476
 hydrochlorate, 476, 787
 Hematoblasts, 619
 Hematochyluria, 676
 Hematocrit, 432
 Hematogenous albuminuria, 294
 albumosuria, 309
 urobilinuria, 283
 Hematoglobulin, 470
 Hematoidin in the blood, 477
 in sputum, 11, 13
 in urine, 362
 Hematopoietic organs, 428, 621
 Hematoporphyrin in blood, 476
 in feces, 116
 in stains, 787
 in urine, 362
 Hematoporphyrinuria, 362
 Hematuria, 389
 angioneurotic, 390
 constitutional, 390
 essential, 390
 extra-renal, 390
 functional, 390
 idiopathic, 390
 renal, 390
 Hemin, 476, 787
 Hemochromogen, 471
 Hemoclastic crises, 617
 Hemoconien, 621
 Hemocytometer of Durham, 561
 of Oliver, 562
 of Thoma-Zeiss, 549
 Hemoglobin in blood, 469
 amount of, 469
 derivatives of, 470
 estimation of, 477
 properties of, 469
 variations of, 490
 in sputum, 3, 5, 11, 13
 in urine, 310, 362
 tests for, 312
 quotient, 491
 value, 491
 Hemoglobinemia, 310
 Hemoglobinemic degeneration, 592
 Hemoglobinometer of Dare, 485
 of Oliver, 487
 of Tallqvist, 489
 Hemoglobinuria, 310, 362
 paroxysmal, 311
 significance of, 310
 tests for, 312
 Hemolysins, 699
 Hemolysis, 699, 722
 Hemolytic anemia, 634
 Hemometer of Fleischl-Miescher, 482
 of Sahli, 486
 Hemophilia, renal, 390
 Hemoptysis, 3
 Hemo-renal index, 504
 Hemorrhage, anemia due to, 632
 occult, 116
 Hemorrhagic exudate, 793
 nephritis, 390
 Hemosiderin, 11, 477
 Hepatic insufficiency, 134, 242, 316, 338
 Hepatogenous albumosuria, 309
 urobilinuria, 283
 Herman-Perutz reaction, 771
 Herpes tonsurans, 179
 Heteroalbumosuria, 305
 Heterochylia, 96
 Heteroxanthin, 265
 Hexamitus duodenalis, 150
 Hexone bases, 90, 98, 273
 High ratio organisms, 854
 Hippuric acid in urine, 275, 380
 test of renal function, 406
 Hirschfeld and Klinger's test, 773
 Histidin, 273
 Histoplasma capsulatum, 674
 Histoplasmosis, 674
 Hodgkin's disease, 641
 Hoffman's bacillus, 44, 849
 Homogentisic acid, 367
 Hopkin's method for uric acid, 259
 Hoppe-Seyler's colorimetric pipet, 478
 Howell's immature nucleated reds, 585
 mature nucleated reds, 585
 Huppert-Messinger method for acetone, 356
 Hyaline casts, 391
 Hydatid cysts, 156, 810
 Hydatidiform degeneration, 425
 Hydræmia, 431
 Hydrobilirubin in stools, 115
 Hydrocele agar, 848
 fluid, 809
 Hydrochloric acid in gastric juice, 55
 amount of, 75
 combined, 78
 deficit, 80
 estimation of, 74
 free, 72
 physiologically active, 79
 tests for, 73
 Hydrogen ion concentration, 437, 448
 of blood, 437, 448
 of culture media, 840
 of gastric juice, 72
 of saliva, 38
 of spinal fluid, 812
 of urine, 198
 sulphid in gastric contents, 90
 in urine, 225
 Hydronephrosis, 809
 Hydrops folliculorum Graafii, 807
 Hydroquinone-acetic acid, 367
 Hydruria, 192
 Hymenolepis diminuta, 155
 flavopunctata, 155
 murina, 154
 nana, 154
 Hypalbuminosis, 493
 Hyperacidity of gastric juice, 72, 78, 95
 Hyperalbuminosis, 493
 Hyperchlorhydria, 78, 95
 Hyperglycemia, 524
 Hyperinosis, 463
 Hypermotility of stomach, 92

- Hypersecretion of gastric juice, 96
 Hypertonic solutions, 595
 Hyphogenous syphilis, 179
 Hypnosis, 463
 Hypochlorhydria, 77
 Hypostatic albuminuria, 293
 Hypotonic solutions, 595
 Hypoxanthin, 256, 265
- Ice-box fixation, 732
 Idiopathic enterogenous cyanosis, 475
 hematuria, 390
 pentosuria, 341
 Ilosvay's reagent, 39
 Immature nucleated reds of Howell, 585
 Immunity, 701
 Inactivation of serum, 699
 Inanition, anemia due to, 633
 Incubation, 843
 Index, bacillary, 702
 color, 491
 hemoglobin, 491
 McLean's, of urea excretion, 505
 of acid excretion, 199
 of urea excretion, 505
 opsonic, 702
 phagocytic, 702
 volume, 433
 India ink method for spirochæte, 800
 Indican, 284
 Indicanuria, 284
 Indigo blue in urine, 284
 red in urine, 287
 Indirect examination of gastric contents, 94
 Indol-acetic acid, 288
 Indoxyl-potassium sulphate in urine, 284
 Induced glycuronic acid test, 348
 Infectious diseases, blood in, 646
 jaundice, 683
 Influenza, bacillus of, 28, 850
 sputum in, 36
 Infusoria, in feces, 151
 in sputum, 32
 in urine, 402
 Inorganic constituents of blood, 535
 of urine, 208
 Inoscopy, 795
 Inosite in urine, 347
 Insecta, 176
 Insufficiency, digestive, 121
 glomerular, 413, 425
 hepatic, 134, 242, 316, 338
 motor, 91
 renal, 413, 425
 tubular, 413, 425
 Intermittent albuminuria, 291
 Intestinal concretions, 126
 digestion, 110
 juices, 110
 obstruction, 121
 parasites, 142
 sand, 126
 Iodid of potassium test, of absorptive power, 93
 of renal function, 406
 Iodoform test for acetone, 354
 for lactic acid, 83
 Iodophilia, 605
- Iron in the blood, 546
 in urine, 231
 Irritation forms of leucocytes, 604
 Isoagglutinins, 777
 Isohemolysins, 777
 Isomaltose in urine, 347
 Isotonicity of red cells, 594
 Isotonic solutions, 594
 Itch parasite, 175
- Jaeger and Heubner's diplococcus, 816
 Jaffe's test for creatinin, 269
 for indican, 285
 von Jaksch's anemia, 630
 Jansky's blood groups, 779
 Japanese liver-fluke, 172
 Jaundice, blood in, 683
 sputum in, 4
 urine in, 196, 363
 Jecorin, 523
 Jenner's stain, 575
 Jigger, 178
 Jolles' ferrometer, 546
 Jousset's fluid, 795
 Juice, gastric, 64
 intestinal, 110
 Justus' test for syphilitic blood, 656
- Kahler's disease, 306
 Kala-azar, blood in, 674
 parasite of, 674
 Karyomorphism of neutrophiles, 600
 Kastle and Loevenhart's method for lipase, 279
 Kathrein's test for bile pigments, 364
 Kelling's test for lactic acid, 82
 Kendall and Day's method for typhoid bacilli, 141
 Kendall and Ryan's double sugar medium, 141
 Ketosis, 351
 Kidney, abscess of, 386
 acute inflammation of, urine in, 193, 195, 205, 209, 216, 234, 290
 amyloid disease of, urine in, 192, 195, 281, 295, 304
 cancer of, 389, 398
 chronic inflammation of, urine in, 193, 195, 205, 209, 216, 295
 echinococcus cysts of, 402
 hemorrhagic lesions of, 389
 hydronephrotic cysts of, 809
 malignant disease of, 389, 398
 stones, 402
 suppurative lesions of, 386
 syphilitic disease of, 294
 tubercular, 387, 399
 Kjeldahl's method for nitrogen, 235
 Klebs-Löffler bacillus, 42, 848
 Knop-Hüfner method for urea, 243
 Koch's bacillus, 18, 142, 399, 695, 795
 comma bacillus, 68, 138, 864
 tuberculin, 18, 704
 Koch-Weeks bacillus, 52
 Kohlrausch's method for electric conductivity, 408, 466
 Kolmer's Standardized Wassermann Test, 739
 Krabbea grandis, 157

- Kramer and Tisdall's method for calcium in blood, 545
 Kreatin (see Creatin), 266, 271, 521
 Kreatinin (see Creatinin), 266, 518
- Lab, 70, 88
 Labor, albuminuria following, 291
 Lactic acid in blood, 529
 in gastric contents, 81
 in carcinoma, 99
 significance of, 81
 tests for, 82
 in urine, 276
 Lactose in milk, 832
 in urine, 345
 test for renal function, 406
 Lactosuria, 345
 Lactose-litmus agar, 842
 Laiose, 341
 Laking of blood, 436, 594
Leishmania intestinalis, 150
 Landau's test, 771
 Lange's colloidal-gold test, 821
 Large lymphocytes, 597
 mononuclear leucocytes, 598
 Larvæ in aural secretions, 51
 in feces, 161, 162, 166
 Laveran's malarial organism, 659
 Layers of sputum, 5
 Lead, anemia due to, 635
 basophilia in poisoning, 593
 Lecithin globules in semen, 418
 Legal's test for acetone, 354
 Leiner's test for casein, 123
 Leishman-Donovan bodies, 674
 Le Nobel's test for acetone, 354
 Leone's test for albumin, 301
 Leo's method for chymosin, 88
 Leprosy, bacillus of, in sputum, 24
 blood in, 656
 Leptodera intestinalis et stercoralis, 160
 Leptospira icterohemorrhagica, 684
 icteroides, 681
 Leptothrix buccalis, 41
 in sputum, 15
 Leptus autumnalis, 175
 Leube's test of gastric motility, 92
 Leucin in sputum, 14
 in urine, 272, 378
 Leucocytes, 597
 appearance of, 597
 basophilic, 602
 counting of, 559
 degenerated forms of, 604
 differential counting of, 605
 eosinophilic, 601
 ferments of, 548
 formation of, 429
 functions of, 618
 granules in, 599, 601, 602
 in blood, 597
 in exudates, 804
 in feces, 128
 in gastric contents, 67
 in milk 836
 in sputum, 10
 in urine, 386
 irritation forms, 604
 karyomorphism of, 600
 Leucocytes, large mononuclear, 598
 lymphocytes, 597
 mast-cell, 602
 myelocytes, 603
 neutrophilic, 599
 number of, 607
 oxyphilic, 601
 pigmented, 603
 polymorphonuclear, 599
 small mononuclear, 597
 splenocytes, 598
 transition forms of, 604
 types of, 597
 variations in number of, 607, 617
 Leucocytic crystals in sputum, 14
 Leucocytometer, 551
 Leucocytosis, 607
 agonal, 612
 antemortem, 612
 cachectic, 611
 eosinophilic, 615
 infectious, 610
 inflammatory, 610
 mast-cell, 616
 mixed, 613
 of digestion, 608
 of pregnancy, 609
 of the new-born, 610
 polymorphonuclear, 608
 post-hemorrhagic, 611
 therapeutic, 612
 Leucohydrobilirubin in feces, 115
 Leucopenia, 617
 Leucorrhea, 422
 Leucourobilin, 115
 Leukanemia, 630
 Leukemia, 636
 acute, 640
 lymphatic, 639
 mixed, 636
 splenomyelogenous, 636
 Levaditi and Manouélian's method for staining spirochete, 801
 Levulose in urine, 338
 determination of, 339
 recognition of, 339
 significance of, 338
 tests for, 339
 Levulosuria, 338
 Levy, Rowntree and Marriott's test for ion concentration of blood, 453
 Lieben's test for acetone, 354
 Lientery, 121
 Limitations of blood examinations, 788
 Limnæa truncatula, 171
 Lipacidemia, 530
 Lipaciduria, 276
 Lipase in gastric juice, 70, 89
 in pancreatic juice, 110
 in urine, 279
 Lipemia, 529, 644
 Liplawsky's test for diacetic acid, 359
 Lipuria, 381
 Liquor sanguinis, 435
 Lithemic diathesis, 258
 Litmus-glucose agar, 842
 lactose agar, 842
 milk, 843
 Liver, abscess of, sputum in, 36

- Liver, insufficiency of, 134, 242, 316, 338
 Lobar pneumonia, blood in, 647
 chlorids in urine of, 210
 organism of, 26, 34
 sputum in, 34
 Lochia alba, 424
 cruenta, 424
 rubra, 424
 serosa, 424
 Löffler's antiformin method, 18
 methylene blue, 21
 Lohnstein's saccharometer, 336
 Long's coefficient, 204
 Lordotic albuminuria, 292
 Low ratio organisms, 854
 Luetin reaction, 706
 Lumbar puncture, 811
 Lung, abscess of, sputum in, 36
 flake in sputum, 32
 inflammation of, 34, 210, 677
 stones, 9
 Lymphatic leukemia, 639
 pseudoleukemia, 641
 Lymphemia, 639
 Lymphocytes, 597
 Lymphocytosis, 614
 Lymphopenia, 614
 Lymphosarcoma, 642
 Lysins, 699
 Lytic action, 699

 Macrocytes, 583
 Macrocythemia, 583
 Macrocytosis, 583, 628
 Macrogamete, 662
 Magnesium ammonium phosphate in feces,
 128
 in sputum, 14
 in urine, 383
 phosphate in urine, 214, 383
 salts in urine, 229
 soaps in feces, 124
 Malaria, blood in, 670
 fresh blood in, 660
 mosquito theory of, 659
 parasites of, 660, 663, 664
 estivo-autumnal, 663, 667
 quartan, 663, 667
 tertian, 660, 666
 stained smears in, 665
 Malarial pigment, 477
 Male secretions, 416
 Malfatti's method for ammonia, 255
 Malignant disease, blood in, 656
 gastric juice in, 98
 urine in, 389, 398
 lymphoma, 641
 pleurisy, 806
 Mallory's stain for Negri bodies, 186
 Malone's test for pregnancy, 273
 Maltose in urine, 346
 Mammary secretions, 825
 Maragliano's endoglobular degeneration,
 592
 Marchal's test for bile pigment, 364
 Marriott's method for alkali reserve of
 blood, 455
 Marshall's method for urea, 247
 Martius and Lüttke's method for HCl, 78

 Marx's fluid, 783
 Mast-cell granules, 602
 leucocytosis, 616
 Masturbators, albuminuria of, 293
 Mature nucleated reds of Howell, 585
 May-Grünwald stain, 575
 McCrudden's method for calcium, 230
 McLean's Index of urea excretion, 505
 Meals, test, 60
 Measles, blood in, 651
 Medicinal leucocytosis, 612
 Medico-legal aspects of blood, 783
 of semen, 419
 Megaloblasts, 585
 Megalocytes, 583
 Megalogastria, 92
 Megastoma entericum, 150
 intestinale, 150
 Melanin, 196, 366, 477
 Melanogen, 196
 Melanuria, 366
 Meltzer-Lyon method, 109
 Membranous dysmenorrhea, 424
 enteritis, 120
 ureteritis, 280
 Meningeal fluid, examination of, 810
 Meningitis, epidemic cerebrospinal, 815
 tubercular, 816
 Meningococcus of Bonome, 816
 of Weichselbaum, 815, 871
 Menstruation, 424
 Messinger method for acetone, 356
 Metalbumin in ovarian cysts, 808
 Metamyelocytes, 600
 Methemoglobin, 471
 Methylene azure stains, 576
 blue in urine, 197, 370
 stains, 21, 575
 Methylphenylsazon, 339
 Methyl red test, 854
 Methylxanthin, 266
 Mett's method for pepsin, 86
 Meyer's test for blood, 785
 Microblasts, 585
 Micrococcus catarrhalis in sputum, 17, 873
 tetragenus in sputum, 17
 Microcytes, 582
 Microgametes, 662
 Microgametocytes, 662
 Microscopy of blood, 563
 of exudates, 803
 of feces, 127
 of gastric contents, 68
 of milk, 834
 of semen, 417
 of sputum, 9
 of urine, 371
 Microsporon Audouini, 180
 furfur, 180
 minutissimum, 182
 scorteum, 179
 Miescher's hemoglobinometer, 482
 Milk, 825
 appearance of, 827
 ash of, 828
 bacteriology of, 834
 coagulation of, 828
 composition of, 826
 cow's, 827

- Milk, curds in stools, 123
 -curdling ferment, 70, 88
 fat of, 831
 human, 826
 lactose of, 832
 microscopy of, 834
 preservatives in, 833
 properties of, 826
 protein of, 829
 reaction of, 828
 specific gravity of, 827
 sugar of, 832
 total solids, 828
- Milky zone, 661
- Mineral acidity of urine, 200
- Minot's method for grouping blood, 781
- Mintz's method for free HCl, 74
- Mitchell's test for alkaline carbonates, 228
- Mitochondria, 584
- Mixed infection in tuberculosis, 23
 leucocytosis, 613
- Molds in aural secretions, 51
 in buccal secretions, 39
 in sputum, 17
- Möller's method for spores, 847
- Monocalcium phosphate in urine, 381
- Monocercomonas hominis, 149
- Monochromatophilia, 591
- Mononuclears, basophile, 604
 eosinophile, 603
 large, 597
 neutrophile, 603
 small, 597
- Moore and Wilson's test for alkalinity, 437
- Morax-Axenfeld diplobacillus, 52
- Mörner-Sjöqvist method for urea, 246
- Mörner's mucin-like bodies in urine, 280
 test for tyrosin, 380
- Morning sputum, 1
 urine, 191
- Moro's tuberculin reaction, 704
- Morphology of blood, 563
 of blood-forming organs, 621
- Mosquito anopheles, 659
 culex, 681
 cycle of malarial parasites, 669
 stegomyia, 681
 theory of malaria, 659
 of yellow fever, 681
- Moss' blood groups, 779
 method for determining, 781
- Motility of intestine, 112
 of stomach, 91
 detection of, 92
 types of, 91
- Motor insufficiency, 91
- Moults, 174
- Mouth, inflammation of, 41
 catarrhal, 41
 gonorrheal, 45
 mycotic, 45
 ulcerative, 44
 ulceromembranous, 44
 secretions of, 38
- Much's method for tubercle bacilli, 22
- Mucin in ovarian cysts, 808
 in sputum, 3
 in urine, 280
- Mucinophiles, 603
- Mucoid material in urine, 384
 sputum, 5
- Mucopurulent sputum, 5
- Mucor in sputum, 17
- Mucous corpuscles, 384
 threads in urine, 388, 797
- Mucus in feces, 119
 appearance of, 119
 detection of, 119
 significance of, 120
 in gastric contents, 66, 68, 98
 in sputum, 5
 in urine, 280
- Müllern's blood stain, 572
- Murexid test, 374
- Muscle fibers in feces, 121
 in gastric contents, 66
 in sputum, 12
- Mycelial casts in sputum, 8
- Myelemia, 636
- Myelin granules in sputum, 11
- Myeloblasts, 598
- Myelocytes, 603
 basophile, 604
 eosinophile, 603
 neutrophile, 603
 Cornil's, 603
 Ehrlich's, 603
- Myelocytosis, 614
- Myelogenous leukemia, 636
- Myeloid leukemia, 636
- Myelopathic albumosuria, 306
- Myxedema, blood in, 646
- Myxococcidium stegomyiae, 681
- Myxoid cyst of ovary, 808
- Myxoma of the placenta, 425
- Nakayama's test for bile pigments, 365
- Naphthoresorcin test, 349
- Nasal secretion, 48
 bacteria in, 49
 composition of, 48
 concretions in, 50
 pathology of, 49
 spinal fluid in, 50
- Negri bodies, 185
- Neisser's diplococcus, 795, 869
 stain for diphtheria bacillus, 42
- Nematodes in feces, 158, 173
 in urine, 402
- Nephritic albuminuria, 294
 hematuria, 389
 oliguria, 193
 pleurisy, 807
- Nephritis, acute, 193, 195, 205, 209, 216, 234, 294
 albuminuria of, 294
 chronic diffuse, 205, 209, 216, 295
 interstitial, 193, 197, 295
 parenchymatous, 193, 195, 295
 hemorrhagic, 389
 suppurative, 386
 syphilitic, 294
 unilateral, 412
- Nervous dyspepsia, 97
 type of albuminuria, 294
 of polyuria, 192
- Neubauer and Fischer's test for gastric carcinoma, 101

- Neuberg and Wohlgemuth's method for pentose, 344
- Neuberg's test for glycuronic acid, 350
for levulose, 339
- Neusser's granules, 600
- Neutral calcium phosphate in urine, 381
dyes, 571
stains, 571
sulphur in urine, 225
- Neutrophile cells, 599, 603
granules, 599
- Neutrophilic karyolobism, 600
- New-born, albuminuria of, 291
leucocytosis of, 610
- Night urine, 191
- Nikiforoff's method of fixation of smears, 569
- Nitric acid test for albumin, 297
- Nitrites in saliva, 39
- Nitrogen of sputum, 2
of urine, 231
partition of blood, 495
of urine, 232
- Nitrogenous balance, 231
bodies in blood, 495
in exudates, 792
in feces, 130
in gastric contents, 100
in milk, 829
in sputum, 2, 5
in transudates, 791
in urine, 231
allantoin, 276
alloxyproteic acid, 225, 275
amino-acids, 272
ammonia, 250, 516
creatinin, 266, 518
hippuric acid, 275, 380
oxyproteic acid, 275
purin bases, 265
total, 231, 495
undetermined, 272
urea, 241, 501
uric acid, 255, 495
equilibrium, 231
- Nitroprussid test for acetone, 354
- Nocht's malarial stain, 578
- Noguchi's antigen, 726
butyric acid test, 818
lutein reaction, 706
method for protein, 819
for spirochete, 679, 801
modification of Wassermann test, 733
- Nondiffusible alkalinity of blood, 437
- Nonne's test of cerebrospinal fluid, 820
- Nonprotein nitrogen of blood, 495
- Normal feces, 105
salt solution, 595
- Normoblasts, 584
- Normocytes, 582
- Nose, secretions of, 48
- Nubecula, 194
- Nubecular threads, 194, 395
- Nucleated red cells, 584
Howell's immature, 585
mature, 585
- Nuclein bases, 130, 206, 265, 376
- Nucleinic acid, 258, 280
- Nucleo-albumin in urine, 280
- Number of blood plates, 620
of leucocytes, 607
of red cells, 587
of stools, 112
of tubercle bacilli in sputum, 24
- Nummular sputum, 5, 6
- Nutrient agar, 841
broth, 839
gelatin, 841
- Nutrition, effect of, on blood, 588, 633
- Nycturia, 191
- Nylander's test for glucose, 324
- Obermayer's test for indican, 286
- Obermeier's spirillum, 671
- Obtaining bile, 109
blood, 434, 727
exudates, 791
gastric contents, 56
- Occult blood in feces, 116
- Ochronosis, 368
- Octomitus hominis, 150
- Odor of blood, 437
of exudates, 792
of feces, 114
of gastric contents, 65
of sputum, 4
of urine, 197
- Oidiomycosis, 45
- Oidium albicans, 17, 45
- Oil test-breakfast, 108
- Oligemia, 430
- Oligochromemia, 491
- Oligocythemia, 589
- Oliguria, 193
- Oliver's hemocytometer, 562
hemoglobinometer, 487
method for typing pneumococci, 28
test for bile acids, 366
- Oöcyst, 669
- Oökinet, 669
- Operation, blood after, 643
- Opisthorchis felineus, 172
sinensis, 172
- Oppler-Boas bacillus, 69
- Opsonic index, 702
- Opsonins, 701
- Optical activity of carbohydrates, 333
of conjugated glycuronates, 334
of glycuronic acid, 334
of urine, 205
- Oral secretions, 38
- Orcein stain, 12
- Orcin test for pentose, 343
- Organic acidity of urine, 200
acids in gastric contents, 8c
in urine, 276
- Organized sediments in urine, 384
bacteria, 398
blood cells, 389
casts, 391
epithelial cells, 384
mucoid material, 384
parasites, 402
pus cells, 386
spermatozoa, 398
tissue fragments, 398
- Origin of casts, 391
of leucocytes, 429

- Origin of red cells, 428
 Orthostatic albuminuria, 292
 Orthotic albuminuria, 292
 Osler's disease, 590
 Osmotic pressure of blood, 464
 of urine, 407
 Otomycosis, 51
 Ova in feces, 143, 167
 in sputum, 32
 in urine, 402
 of anopheles, 659
 of intestinal parasites, 167
 Ovarian cysts, 807
 colloid, 808
 dermoid, 809
 myxoid, 808
 papillary, 809
 serous, 807
 Ovoids in malarial blood, 665
 Oxalate of calcium in sputum, 14
 calculi, 403
 in urine, 277, 376
 Oxalic acid in urine, 277
 amount of, 277
 determination of, 278
 origin of, 277
 variations of, 277
 Oxaluria, 277
 Oxaluric acid, 277
 Oxid of bismuth in feces, 115
 β -oxybutyric acid in urine, 359
 determination of, 360
 significance of, 359
 tests for, 360
 Oxygen-binding capacity of blood, 478
 Oxygen unsaturation, 547
 Oxyhemoglobin, 470
 p-oxyphenyl- α -amino-propionic acid, 379
 Oxyphilic cells, 601
 granules, 601
 Oxyproteic acid, 207, 275
 Oxyuris vermicularis, 159
 Ozena, 50

 Palpation, albuminuria due to, 291
 Paludism, 659
 Pancreatic cysts, 810
 disease, feces in, 122
 fluid, 810
 juice, 110
 composition of, 110
 ferments of, 110
 insufficiency of, 122
 Panoptic staining, 572, 575
 Papillary cysts of the ovary, 809
 Pappenheim's amblyochromatic erythro-
 blasts, 585
 heteroplasic promyelocytes, 603
 method for tubercle bacillus, 22
 stain for blood smears, 575, 578
 trachyochromatic erythroblasts, 584
 Paracresol, 367
 Paragonimus westermanii, 32
 Paramecium coli, 151
 Paramucin, 808
 Parasites, anemia due to, 634
 eosinophilia due to, 615
 in blood, 658
 in feces, 142
 Parasites, in sputum, 32
 intestinal, 142
 in urine, 402
 malarial, 659
 of the skin, 174
 Parasitology of the blood, 658
 of the feces, 142
 of the skin, 174
 Paratyphoid bacillus, 140, 690
 Paraxanthin, 266
 Parenchymatous nephritis, acute, 193, 195,
 205, 209, 216, 234, 295
 chronic, 193, 194, 295
 Parhemoglobin, 470
 Parovarian cysts, 809
 Paroxysmal hemoglobinuria, 311
 polyuria, 191
 Pathogenic bacteria in blood, 687
 in exudates, 795
 in feces, 136
 in gastric contents, 69
 in milk, 834
 in sputum, 18
 in urine, 398
 Pea-soup stools, 139
 Pediculus capitis, 176
 pubis, 176
 vestimenti, 176
 Penicillium glaucum, 17
 Pentose in urine, 341
 determination of, 343
 significance of, 341
 tests for, 342
 Pentosuria, alimentary, 341
 essential, 341
 idiopathic, 341
 intrinsic, 341
 Penzoldt and Faber's test, 93
 Pepsin in gastric juice, 71, 84
 activity of, 84
 detection of, 85
 determination of, 86
 significance of, 85
 in urine, 279
 Pepsinogen, 84
 Peptic glands, 54
 Peptone in the blood, 494
 in gastric contents, 89
 in urine, 310
 Peptonuria, 310
 Perez' bacillus, 50
 Perforating empyema, sputum in, 37
 Pericardial fluid, 805
 Perinuclear granules of Neusser, 600
 Periodic albuminuria, 292
 polyuria, 191
 Peritoneal exudates, 807
 composition of, 807
 cytology of, 807
 Permeability of red cells, 594
 renal, 406
 Pernicious anemia, 627
 Pertussis, blood in, 653
 organism of, 29, 851
 Pessary forms of red cells, 580
 Petroff's method for tubercle bacilli, 19
 Pettenkofer's test, 366
 Petzetaki's test for tuberculosis, 371
 Pfeiffer's bacillus, 28, 36, 850

- Pfeiffer's bacteriolytic test, 865
 Phagocytic cells, 4, 701
 index, 702
 Phagocytosis, 701
 Pharyngomycosis leptothrica, 41
 Phenol in feces, 106
 in urine, 367
 Phenolphthalein test, 785
 Phenolsulphonaphthalein test, 412
 Phenolsulphuric acid, 367
 Phenoltetrachlorophthalein test, 134
 Phenylglucosazon, 326
 Phenylhydrazine test for glucose, 326
 Phloridzin test, 412
 Phloroglucin test for pentose, 342
 vanillin test for HCl, 73
 Phosphates, calcium, 214, 229
 in blood, 540
 in sputum, 14
 in urine, 214
 magnesium, 214, 229, 383
 magnesium-ammonium, 14, 128, 383
 triple, 14, 128, 383
 Phosphatic calculi, 405
 diabetes, 217
 sediments in urine, 382, 383
 Phosphaturia, 214, 382
 Phosphorus containing proteins, 280
 poisoning, blood in, 635
 urine in, 242
 Phthirus inguinalis, 176
 Phthisis, blood in, 655
 hemoptysis in, 3
 melanotica, 11
 sputum in, 33
 stone-cutters', 4
 Physiological albuminuria, 289
 glycosuria, 313
 salt solution, 595
 variations in blood cells, 587, 608
 Physiologically active HCl, 78
 Pylorus intestinalis, 166
 Pigment, bile, in blood, 534
 in feces, 116, 125
 in gastric contents, 66, 68
 in sputum, 3
 in urine, 363
 blood, in feces, 116
 in gastric contents, 66, 68, 90
 in sputum, 3, 5, 11, 12, 14
 in urine, 362
 coal, in sputum, 4, 37
 in leucocytes, 4, 661, 663, 664
 in red cells, 571, 660
 of blood, 469
 of urine, 281, 362
 Pin worm, 159
 Pineapple test, 84
 Piroplasma hominis, 674
 Piroplasmosis, 674
 von Pirquet's tuberculin reaction, 705
 Placenta cells, 425
 Plague bacillus, 29
 Plasma, 432
 chlorids, 535
 Plasmodium falciparum, 664
 quotidianum, 663
 malariae, 663
 precox, 664
 Plasmodium tenue, 660
 vivax, 660
 variety minuta, 660
 Platelets, blood, 619
 Platodes in feces, 151
 Plehn's karyochromatophilic granules, 666
 Plethora, cellular, 431
 serous, 431
 true, 431
 vera, 431
 Pleuritic effusions, 805
 cytology of, 805
 withdrawal of, 791
 Plugs, Dittrich's, 7
 prostatic, 418
 Pneumococcus of Fraenkel, 26, 34, 692, 865
 pleurisy, 806
 types of, 26
 Pneumoliths, 9
 Pneumonia, blood in, 647
 chlorids in urine in, 208
 organism of, 26, 34, 692, 865
 sputum in, 34
 urine in, 208
 Pneumonoconioses, 37
 Pneumomycosis aspergillina, 17
 Poikilocytes, 583
 Poikilocytosis, 583
 Poisons, blood, 635
 Polariscope, 333
 Polariscopic method for glucose, 333
 Poliomyelitis, acute anterior, 817
 Polychromasia, 591
 Polychromatophilia of Gabritschewsky, 592
 of Maragliano, 593
 Polychrome dyes, 575
 Polychromemia, 590
 Polycythemia, 590
 Polyglobulia, 590
 Polymorphonuclear basophiles, 602
 eosinophiles, 601
 neutrophiles, 599
 neutrophiliosis, 607
 Polynucleosis, 607, 805
 Polyplasmia, 431
 Polyuria, 191
 epicritic, 192
 paroxysmal, 192
 periodic, 192
 Poor, anemia of the, 633
 Pork tape-worm, 153
 Posterior urethritis, 388
 Post hemorrhagic anemia, 632
 infectious albuminuria, 293
 leucocytosis, 611
 Postural albuminuria, 292
 Potassium acid urate sediment, 375
 ferrocyanid test for albumin, 300
 iodid test, 93
 for renal function, 406
 of blood, 467
 of urine, 228
 sulphocyanate in saliva, 38
 Precipitinophore, 701
 Precipitin test for blood, 718
 for semen, 420
 Precipitins, 701
 Pregnancy, albuminuria of, 291
 ammonia in urine of, 251

- Pregnancy, anemia of, 633
 blood in, 609
 leucocytosis of, 609
 sero-diagnosis of, 757
 urine test in, 273
 Preparation of blood smears, 565
 culture media, 839
 vaccines, 874
 Preservation of urine, 189
 Primary anemia, 624
 pernicious anemia, 627
 proteoses, 305
 tubercular pleurisy, 805
 Products of gastric digestion, 89
 of intestinal digestion, 111
 Progressive pernicious anemia, 627
 Promyelocytes, 603
 Propepsin, 55, 84
 Prostatic casts, 418
 fluid, 416
 plugs, 418
 secretion, 416
 Prostatitis, 388
 Prostatorrhea, 418
 Protalbumose, 305
 Protein in blood, 492
 in exudates, 792
 in feces, 123
 in gastric contents, 89, 100
 in milk, 829
 in sputum, 5
 in urine, 289
 quotient of serum, 494
 Proteoses in urine, 305
 Prothrombase, 460
 Protoryxomyces coprinarius, 149
 Protozoa in blood, 658
 in feces, 144
 in gastric contents, 70
 in sputum, 32
 in urine, 402
 Provocative Wassermann test, 754
 Prowazek-Greeff trachoma bodies, 53
 Prune-juice sputum, 3, 34
 Pseudo casts, 396
 diphtheria bacillus, 44, 849
 elastic tissue, 12
 gall stones, 126
 globulin, 304
 hemoglobin, 470
 leukemia, 641
 infantum, 630
 mucin, 808
 nucleation, 592
 parasites, 166
 rhabditis stercoralis, 160
 Psilosis, 45
 Ptomaines in feces, 131
 in urine, 377
 Ptyalin, 38, 39, 89
 Ptyalism, 40
 Puberty, albuminuria of, 293
 Puerperal infection, 423
 Pulex irritans, 178
 penetrans, 178
 Pulmonary actinomycosis, 30
 gangrene, 36
 hemorrhage, 3
 tuberculosis, 33
 Punctate basophilia of Grawitz, 593
 Purdy's method for albumin, 303
 for chlorids, 214
 for glucose, 331
 for phosphates, 222
 for sulphates, 228
 Purification of picric acid, 262
 Purin bases in urine, 206, 265, 376
 Purpura hemorrhagica, blood in, 633
 Purpurin, 282
 Purulent exudates, 794
 sputum, 5
 urine, 386
 Pus casts, 394
 cells in feces, 121
 in gastric contents, 69
 in sputum, 10
 in urine, 386
 enumeration of, 389
 significance of, 387
 tests for, 388
 Putrescin, 131, 377
 Putrid bronchitis, 35
 exudates, 794
 Pycnometer, 203, 458
 Pycnotic nucleus, 585
 Pyelitis, urine in, 386
 productiva, 280
 Pyelonephritis, 386
 Pyloric glands, 54
 stenosis, 93, 99
 Pyogenic albumosuria, 309
 Pyonephrosis, 386
 Pyorrhea alveolaris, 46
 Pyrocatechin, 367
 Pyuria, 386

 Quantity of blood, 429
 of gastric juice, 64
 of urine, 190
 Quartan malarial parasite, 663, 667
 asexual cycle of, 663
 sexual cycle of, 669
 Quotient, albumin, 304, 494
 protein, 304, 494
 volume, 433

 Rat-bite fever, 686
 Ratio of N to Cl output, 208
 to P_2O_5 output, 216
 to SO_3 output, 223
 Ray fungus, 30
 Reaction of blood, 437
 of feces, 129
 of gastric contents, 64, 70
 of milk, 828
 of spinal fluid, 812
 of sputum, 3
 of urine, 198
 Reactivity of blood, 437
 Receptors, 697
 Rectum, blood in cancer of, 116
 Red cells (see Erythrocytes), 580
 in exudates, 793
 in feces, 116, 128
 in gastric contents, 66, 68, 90
 in sputum, 3, 5, 11
 in suspected stains, 783
 in urine, 389

- Red, indigo, in urine, 287
 jigger, 178
 sputum, 3
 Refraction coefficient of serum, 403
 Rehfuß fractional method, 58
 Reichmann's disease, 96
 Relapsing fever, 671
 Relative concentration, 205
 value of phosphoric acid, 217
 Removal of albumin, 303
 of glucose, 333
 of turbidity, 295
 Renal abscess, 386
 albuminuria, 294
 albumosuria, 310
 aneurism, 390
 calculus, 402
 concretions, 402
 diabetes mellitus, 314, 412
 diagnosis, 406
 epistaxis, 390
 epithelial cells in urine, 384
 functional tests, 406
 hematuria, 390
 hemophilia, 390
 insufficiency, 388
 threshold, 523
 Rennin, 70, 88
 Resistance of red cells, 594
 Resorcin test for free HCl, 73
 Rhabdonema intestinalis, 160
 strongyloides, 160
 Rhamnose in urine, 341
 Rheumatism, blood in, 654
 Rhinitis, 49
 Rhizopoda in feces, 144
 Rice-water stools, 114, 138
 vomitus, 68
 Rickets, blood in, 646
 Rickett's organism of spotted fever, 687
 Riegel's method for chymosin, 88
 test meal, 61
 Rigg's disease, 46
 Ring bodies in red cells, 593
 of Cabot, 594
 worm of the beard, 179
 of the body, 179
 of the scalp, 180
 Ringers' solution, 595
 Rivalta's test for exudates, 792
 Roberts' method for glucose, 337
 test meal, 61
 Roch's test for albumin, 300
 Rocky Mountain spotted fever, blood in, 686
 organism of, 687
 Romanowsky's stain, 575
 Ronchèse's method for ammonia, 255
 Rosacic acid, 282
 Rosenbach's method for bile pigments, 365
 for skatoxyl, 287
 Rosenow's capsule stain, 693
 Rosin's test for bile pigments, 364
 Ross-Jones test of cerebrospinal fluid, 820
 Rot, grinders', 4
 Rouleaux formation, 581
 Round worms in feces, 158
 Rowntree and Geraghty's test, 412
 Rubner's test for lactose, 346
 Rudisch and Kleeberg's method for uric acid, 261
 Rudolf's method for coagulation time, 462
 Ruhemann's uricometer, 264
 Russell and Brodie's coagulometer, 462
 Russo's test for typhoid, 370
 Rusty sputum, 3, 34
 Saccharometer of Einhorn, 336
 of Lohnstein, 336
 Saccharomyces cerevisiæ, 16, 325
 Saccharose in urine, 347
 Sachs-Georgi test, 772
 Sagitula hominis, 166
 Sago-like granules in sputum, 1, 11
 Sahli's desmoid reaction, 94
 hemometer, 486
 test-meal, 62
 Salicylic acid as preservative, 834
 test for gastric motility, 92
 Saliva, 38
 amount of, 38
 bacteria in, 39
 cells in, 39
 chemistry of, 38
 ferments in, 39
 microscopic examination of, 39
 nitrites in, 39
 obtaining of, 40
 pathologic changes in, 40
 potassium sulphocyanate in, 38
 ptyalin in, 38, 39
 Salivary corpuscles, 39
 Salivation, 40
 Salkowski-Ludwig method for uric acid, 260
 Salol test of Ewald and Sievers, 92
 Salomon's test for gastric carcinoma, 100
 Salt glycosuria, 314
 Salzer's test-meal, 62
 Sand flea, 178
 intestinal, 126
 in urine, 402
 renal, 402
 Sanford's method for blood grouping, 781
 Sanguinous exudates, 793
 sputum, 5
 Saprophytes in feces, 136
 in sputum, 14
 in urine, 398
 Sarcinæ in gastric contents, 70
 in sputum, 17
 in urine, 398
 ventriculi, 70
 Sarcoma, blood in, 657
 Sarcoptes scabiei, 175
 Saturation deficit, 80
 Scarlet fever, blood in, 650
 Schær's test for blood, 784
 Scherer's method for albumin, 301
 test for leucin, 379
 Schick reaction, 708
 Schistocytes, 584
 Schistosomum hematobium in blood, 687
 in urine, 402
 Schizogone, 662
 Schizont, 662
 Schlösing's method for ammonia, 252
 Schmaltz' specific gravity tubes, 458

- Schmidt and Strasburger's standard diet, 106
 Schmidt's fermentation method for feces, 133
 Schügner's granules, 666
 Schultze's granular cells, 599, 601
 Sclerostoma duodenale, 164
 Scybala, 113
 Seat worms, 159
 Sebelien's method for protein in milk, 829
 Secondary anemia, 631
 proteoses, 308
 tubercular pleurisy, 806
 Secretin, 110
 Secretion of gastric juice, 55
 of genital organs, 416
 of mammary glands, 825
 of urine, 189
 Sedimentation, 371
 Sediments in urine, 372
 bacteria, 398
 bilirubin, 380
 blood cells, 389
 calcium carbonate, 384
 oxalate, 376
 phosphate, 381
 sulphate, 380
 casts, 391
 cholesterin, 381
 cystin, 377
 epithelial cells, 384
 fat, 381
 hematoidin, 380
 hippuric acid, 380
 indigo, 285
 leucin, 378
 magnesium ammonium phosphate, 383
 phosphate, 383
 mucoid material, 384
 mucous threads, 384
 nubecular threads, 194, 395
 organized, 384
 parasites, 402
 phosphates, 381, 382
 preservation of, 190
 pus cells, 386
 spermatozoa, 398
 tissue fragments, 398
 tyrosin, 379
 unorganized, 374
 urates, 375
 uric acid, 374
 xanthin, 376
 Sedimentum lateritium, 375
 Seliwanoff's test for levulose, 339
 Semen, 416
 chemistry of, 416
 medico-legal aspects of, 419
 microscopic examination of, 417
 pathology of, 418
 precipitin test for, 420
 recognition of stains of, 419
 spermatic crystals in, 416
 spermatozoa in, 417
 Seminal stains, 419
 medico-legal aspects of, 419
 Separate analyses of corpuscles and plasma, 408
 Septic pleurisy, 805
 Sero diagnosis, of gonorrhea, 756
 of pregnancy, 757
 of syphilis, 721
 of typhoid, 711
 of tuberculosis, 756
 Serous cysts of the ovary, 807
 exudates, 792
 plethora, 431
 pleurisy, 805
 sputum, 5
 Serum albumin in blood, 492
 in urine, 289
 diagnosis of syphilis, 721
 of typhoid, 711
 globulin, 304
 determination of, 305
 significance of, 304
 test for, 304
 variations of, 304
 pathology, 696
 reactions, 710
 refraction coefficient of, 493
 special properties of, 696
 Sex, variations of blood cells due to, 587
 cycle of malarial parasite, 669
 secretions, 416
 Shadows of leucocytes, 604
 red cells, 583
 Shaffer's method for acetone bodies, 361
 Shiga's bacillus, 141, 855
 Showers of casts, 392
 Side-chain theory of Ehrlich, 697
 Siderosis, 37
 Signet rings in malarial blood, 661, 665
 Significance of acetoneuria, 350
 of albuminuria, 290
 of albumosuria, 309
 of Bence Jones proteinuria, 305
 of cylindruria, 391, 395
 of free HCl in gastric contents, 76
 of globulinuria, 304
 of glycosuria, 314
 of hematuria, 389
 of lactic acid in stomach, 81
 of leucocytosis, 607
 of levulosuria, 338
 of mucous threads in urine, 384, 395
 of mucus in feces, 120
 of nitrogen-partition of urine, 232
 of β -oxybutyric acid in urine, 359
 of pentosuria, 341
 of pepsin in gastric juice, 85
 of pyuria, 386
 Sjöqvist's method for urea, 246
 Skatoxyl-potassium sulphate in urine, 287
 Skin, blood in diseases of, 616
 parasites of, 174
 Sleeping sickness, blood in, 672
 organism of, 672
 Small-pox, blood in, 652
 Smears, preparation of, 565
 of blood, 565
 of exudates, 797, 799
 of feces, 127
 of pus, 797
 of sputum, 10
 of syphilitic material, 799
 Smegma bacillus in buccal secretions, 40
 in exudates, 798

- Smegma bacillus in sputum, 25
 in urine, 399
 preputii, 798
 Smith's test for bile pigments, 364
 Soaps in feces, 124
 Sodium acid urate, 375
 carbonate as preservative of milk,
 833
 chlorid retention, 209
 in nephritis, 210, 408
 in pneumonia, 209
 in blood, 546
 in urine, 228
 Soft chancre, organism of, 798
 Soluble starch, 39, 89
 Solvents for blood stains, 783
 Specific gravity of blood, 457
 of cerebrospinal fluid, 812
 of exudates, 792
 of milk, 827
 of serum, 457
 of transudates, 791
 of urine, 202
 Specificity of agglutination test, 716
 of complement-fixation test, 750
 of precipitin test, 720
 Spectrophotometer of Hüfner, 478
 Spectroscopic examination, 787
 tests for blood, 470, 787
 Spermatic crystals, 416
 Spermatocoele, 809
 Spermatorrhoea, 398, 418
 Spermatozoa, 398, 417
 Spermin crystals, 14, 416
 Spiegler's test for albumin, 300
 Spirals of Curschmann, 7, 33, 36
 Spirillum of Asiatic cholera, 68, 139, 864
 of Obermeier, 671
 of relapsing fever, 671
 of Vincent, 44
 Spirocheta, bronchialis, 37
 buccalis, 39
 icterohemorrhagica, 684
 microdentium, 39
 morsus-muris, 686
 nodosa, 684
 pallida, 676, 798
 refringens, 678
 Spiroplasma balanitis, 799
 pallidum, 676, 798
 characteristics of, 676, 678
 cultivation of, 679
 in blood, 678
 in exudates, 798
 in tissues, 801
 staining of, 676, 799
 recurrentis, 671
 Spit cups, 2
 Spleen, diseases of, blood in, 629
 removal of, 658
 Splenectomy, blood after, 658
 Splenic anemia, 629
 Splenocytes, 598
 Splenomegaly, blood in, 629
 tropical, 674
 Splenomyelogenous leukemia, 636
 Spore cyst, 669
 Spores, staining of, 846
 Sporoblast, 669
 Sporogone, 662
 Sporogony of malarial parasite, 669
 Sporothrix Schenckii, 184
 Sporotrichosis, 184
 Sporozoa in feces, 149
 Sporozoites, 669
 Spotted fever, organism of, 686
 Sprue, 46
 Sputum, 1
 air in, 4
 albumin in, 5
 amount of, 2
 bacteria in, 14
 biliary pigments in, 3
 blood in, 3, 5, 11
 character of, 4
 cheesy particles in, 6
 chemistry of, 5
 chromogenic bacteria in, 4
 coal pigment in, 4, 37
 coctum, 35
 collection of, 2
 color of, 3
 concretions in, 8
 consistency of, 2
 cotton fibers in, 4
 crudum, 34
 crystals in, 13
 Curschmann's spirals in, 7, 33, 36
 cytology of, 10
 deposition on standing of, 5
 Dittrich's plugs in, 7
 echinococcus membranes in, 9
 elastic tissue in, 12
 epithelial cells in, 11
 extraneous matter in, 9
 fatty acids in, 13
 ferments in, 6
 ferric oxid in, 4
 fibrinous casts in, 8
 flour in, 4
 foreign bodies in, 9
 fundum petens, 4
 heart disease, cells in, 11, 35
 hemoglobin derivatives in, 11, 13
 in abscess of the lung, 36
 in actinomycosis, 30
 in acute bronchitis, 34
 in bronchial asthma, 36
 in broncho-pneumonia, 34
 in chronic bronchitis, 35
 in croupous pneumonia, 34
 in fibrinous bronchitis, 35
 in gangrene of the lung, 36
 in influenza, 36
 in jaundice, 4
 in perforating empyema, 37
 in pneumoconioses, 37
 in pulmonary tuberculosis, 33
 in putrid bronchitis, 35
 leucocytes in, 10
 macroscopic examination of, 6
 microscopic examination of, 9
 morning, 1
 mucin in, 5
 mucoid, 5
 mucopurulent, 5
 myelin granules in, 11
 nitrogen of, 2

- Sputum, nummular, 5, 6
 odor of, 4
 origin of, 1
 parasites in, 32
 prune-juice, 3, 34
 purulent, 5
 pus cells in, 10
 reaction of, 3
 red blood cells in, 11
 sanguinous, 5
 serous, 5
 spit-cups for, 2
 stone dust in, 4
 tenacity of, 3
 types of, 5
- Staining characteristics of tubercle bacillus, 20
 methods, principles of, 70
 of bacteria, 846
 of blood smears, 572
 of casts, 392
 of elastic tissue, 12
 properties of cells, 591
 vital, 579
- Stadie's method for methemoglobin in blood, 471
- Stains, Albert's, 43
 blood, 572
 Bunge and Trantenroth's, 25
 Burri's, 800
 Cunningham's, 576
 dahlia, 602
 Ehrlich's tri-acid, 574
 triple, 574
 eosin hematoxylin, 573
 methylene blue, 575
 Fontana's, 801
 Gabbet's, 22
 Ghoreyeb's, 799
 Giemsa's, 577, 799
 Goldhorn's, 679
 Gram's, 796
 india-ink, 800
 iodine, 602
 Jenner's, 575
 Leiner's, 123
 Leishman's, 576
 Levaditi and Manouelian's, 801
 Löffler's methylene blue, 21
 Mallory's, 186
 May-Grünwald's, 575
 Müllern's, 572
 Neisser's, 42
 Nocht's, 578
 Noguchi's, 801
 orcein, 12
 osmic acid, 124
 Pappenheim's for blood, 574, 578
 for tubercle bacillus, 22
 polychrome, 575
 Romanowsky's, 575
 safranin, 797
 scharlach R, 124
 seminal, 419
 sudan III, 124
 thionin, 121, 578
 Tribondeau's, 800
 Tunnicliff's, 799
 Türk's iodine, 602
- Stains, Unna-Tänzer's, 12
 Van Gieson's, 186
 Warthin and Starry's, 802
 Weigert's fibrin, 8
 Williams and Lowden's, 186
 Wright's, 576
 Zenoni's, 5
 Ziehl-Neelsen, 20
- Standardized Wassermann test, 739
- Staphylococcus pyogenes in sputum, 29
- Starch in feces, 132
 detection of, 132
 digestion of, 39
 estimation of, 132
- Steatorrhea, 123, 131
- Stegomyia calopus, 682
 fasciata, 681
- Stercobilin, 114, 283
- Sterility, 418
- Sterilization, 838
- Stippled cells, 593, 651
- Stock-vaccines, 874
- Stomach, absorptive power of, 93
 carcinoma of, 98
 contents, 54
 dilatation of, 91, 92
 diseases of, 95
 ectasia of, 92
 fasting, 66
 function of, 91
 histology of, 54
 inflammation of, 97
 motility of, 91
 tube, 56
 ulcer of, 98
 washing, 57, 100
- Stomatitis, catarrhal, 41
 gonorrheal, 45
 mycotic, 45
 ulcerative, 44
 ulceromembranous, 44
- Stone cutters' phthisis, 4
- Stones, bronchial, 8
 gall, 126
 in bladder, 402
 in kidney, 402
 in lung, 9
 intestinal, 125
 in ureter, 402
 in urine, 402
 nasal, 50
 renal, 402
 ureteral, 402
 vesical, 402
- Stools (see Feces), 105
 acholic, 115, 124
 clay-colored, 115
 curds in, 123
 frequency of, 112
 pea-soup, 139
 rice-water, 114, 138
- Strasburger's method for bacteria in feces, 136
- Strauss' test for lactic acid, 82
- Streptococcal sore throat, 45
- Streptococcus epidemicus, 45
 hemolyticus, 694, 866
 mucosus capsulatus, 694, 866
 pleurisy, 806

- Streptococcus pyogenes* in sputum, 29
 in blood, 694, 866
 viridans in blood, 694, 866
Streptothricosis, 16, 30
Streptothrix eppingeri, 15
 muris ratti, 686
Striatula, 166
Strongyloides intestinalis, 160
Strongylus duodenalis, 164
 gigas, 173
 quadridentatus, 164
 renalis, 173
Structural albuminuria, 294
Sudanophiles, 604
 Sugar broth, 841
 Sugar-free broth, 841
Sulphates of urine, 223
 easily split, 223
 ethereal, 224
 preformed, 224
 total, 224
 unoxidized, 223
Sulph-hemoglobin, 475
Sulphhemoglobinemia, 475
Sulphocyanates in saliva, 38
Sulpho-salicylic acid test for albumin, 300
Sulphur compounds in urine, 223
 amount of, 223
 determination of, 226
 neutral, 225
 test for bile in urine, 366
 types of, 223
 variations of, 224
Surgical interference, blood after, 643
Syphilis, albuminuria of, 294
 blood in, 655
 hemoglobin test of Justus in, 666
 organism of, 676, 798
 serum test of Wassermann in, 724
Tabanus striatus fabricus, 674
 Table for examination of calculi, 404
 Gaffky's, 23
Tæniidæ in feces, 153
 tænia ægyptica, 154
 canina, 154
 cucumerina, 154
 cucurbitina, 153
 dentata, 153
 diminuta, 155
 echinococcus, 155
 elliptica, 154
 flavopunctata, 155
 inermis, 153
 lata, 156
 leptocephala, 155
 mediocanellata, 153
 minima, 155
 moniliformis, 154
 nana, 154
 saginata, 153
 solum, 153
 varerina, 155
Tallqvist's hemoglobinometer, 489
 Tape-worms in feces, 151
 Tartar of the teeth, 41
Taurocholic acid, 125, 207, 365
 Taylor's ash-free diet, 210
Teichmann's crystals, 787
 test for blood, 787
 Tenacity of sputum, 3
 Tertian malarial organism 660, 666
 asexual cycle of, 662
 sexual cycle of, 669
 Test meal of Boas, 61
 of Dock, 61
 of Ewald, 60
 of Fischer, 62
 of Riegel, 61
 of Roberts, 61
 of Sahli, 62
 of Salzer, 62
 oil, 108
 renal, 408
 Testicular casts, 418
Thecosoma hematobium, 687
Theobromin, 266
Theophyllin, 266
 Therapeutic measures, effect of, on blood, 589, 612
 Thermolabile substances, 699
 Thermostable substances, 699
 Thionin stain, 121, 578
Thiosulphuric acid in urine, 206
 Third corpuscles of blood, 619
 Thomas and Weber's method for pepsin, 87
 Thoma-Zeiss hemocytometer, 549
 Thorn-apple crystals, 382
 Thread worm, 159
Threads, mucus in urine, 194, 384, 395
 Threshold of chlorid excretion, 536
 of sugar excretion, 523
 Throat cultures, 42
 Thrombase, 460
 Thrush, 45
 Tick fever, 686
 Tide, alkaline, of urine, 201
Timothy bacillus in sputum, 25
Tinea barbæ, 179
 circinata, 179
 favosa, 178
 sycosis, 179
 tonsurans, 180
 versicolor, 182
 Tisdall's method for phosphates in blood, 542
 Tissue, elastic in sputum, 12
 fragments in feces, 127
 in gastric contents, 70
 in sputum, 9
 in urine, 398
 Toisson's fluid, 553
 Tolerance for sugar, 314
 Tollen's orcin test for pentose, 343
 naphthoresorcin test for glycuronic acid, 349
 phloroglucin test, 342
 test for pentose, 342
 Tongue, coating of, 41
 Tonsillitis, leucocytosis in, 611
 Töpfer's method for combined HCl, 79
 for free HCl, 75
 test for free HCl, 73
 Total acidity of gastric juice, 71
 components of, 71
 determination of, 71
 limits of, 72

- Total acidity of urine, 199
 nitrogen of blood, 495
 of feces, 130
 of gastric juice, 89
 in carcinoma, 100
 of urine, 231
 amount of, 231
 determination of, 235
 variations of, 233
 solids of blood, 468
 of feces, 130
 of milk, 829
 of urine, 204, 207
 non-protein nitrogen of blood, 495
 volume of blood, 429
 Towel test for hemoglobin, 442
 Toxemia, hepatic, 278, 316, 338
 intestinal, 131
 renal, 406
 Toxogenic protein decomposition, 234
 Toxoids, 698
 Toxones, 698
 Toxophore, 698
 Trachoma bodies, 53
 Trachyochromatic erythroblasts, 584
 Transfusion, tests before, 777
 Transitional leucocytes, 599
 Transudates, 790
 coagulation of, 791
 obtaining of, 791
 properties of, 791
 Traumatic albuminuria, 293
 Treatment, effect of on Wassermann's test,
 753
 Trematodes, 169
 in feces, 157
 in sputum, 32
 Treponema pallidum (see *Spironema pal-*
 lidum), 676, 798
 Triacid stain of Ehrlich, 574
 of Pappenheim, 575
 Tribondeau's stain, 800
 Trichina spiralis, 162
 Trichinella spiralis, 162
 Trichinosis, 162, 615
 Trichiuris trichiura, 162
 Trichocephalus dispar, 162
 hominis, 162
 mastigodes, 162
 trichiuris, 162
 Trichomonas hominis, 149
 intestinalis, 149
 vaginalis, 402
 in urine, 402
 Trichophyton megalosporon endothrix, 179
 microsporon, 180
 Trichotrachelidæ, 162
 Triple phosphates as calculi, 405
 in sputum, 14
 in urine, 383
 Tripperfaden in urine, 388, 395, 779
 Trombidiosis, 176
 Trombidium irritans, 175
 Trommer's test for glucose, 319
 Tropæolin test for HCl, 74
 Tropical splenomegaly, 674
 Tropics, anemia of, 588
 Trousseau's test for bile pigments, 364
 True albuminuria, 289
 Trypanosoma Brucei, 674
 equiperdum, 674
 Evansi, 674
 Gambiense, 672
 in the blood, 672
 in spinal fluid, 673, 816
 Trypanosomiasis, 672
 Trypsin in feces, 110
 in pancreatic cysts, 810
 in urine, 279
 Tryptophan test, 101
 Tsetse flies, 672
 Tsuchiya's method for albumin, 302
 Tube casts in urine, 391
 Tubercle bacilli in the blood, 695
 in exudates, 795
 inoscopy, 795
 in feces, 142
 in sputum, 18
 culture medium for, 19
 morphology of, 22
 number of, 24
 staining of, 20
 value of examination for, 23
 in urine, 399
 Tubercular meningitis, 816
 pleurisy, 805
 Tuberculin, 18
 reactions, 704
 Tuberculosis, blood in, 655
 complement-fixation test in, 756
 of bladder, 399
 of intestine, 142
 of kidneys, 387
 of lymph glands, 642
 of meninges, 816
 of peritoneum, 807
 of pleura, 805
 pulmonary, blood in, 655
 sputum in, 33
 Tuberculous cystitis, 399
 Tubular insufficiency, 406
 Tumor shreds in feces, 127
 in gastric contents, 70
 in urine, 398
 Tunnel workers' anemia, 166
 Tunnicliff's bacillus, 49
 stain for spirochæte, 799
 Türk's iodine stain, 602
 counting chamber, 552
 Two-glass test, 388
 Types of pneumococci, 26
 Typhoid bacillus in the blood, 689
 in feces, 139
 Drigalski and Conradi's media, 139
 Kendall and Day's media, 141
 in urine, 401
 fever, blood in, 649, 711
 feces in, 139
 Widal reaction in, 711
 pleurisy, 806
 Typhoidin reaction, 708
 Typing of pneumococci, 26
 Tyrosin in sputum, 14
 in urine, 272, 379
 Uffelmann's test for lactic acid, 82
 Uhlenhuth's antiformin method for tubercle
 bacilli, 18

- Ulcer of the stomach, 98
 Ulceromembranous angina of Vincent, 44
 Unaltered bile in feces, 116
 Uncinaria Americana, 165
 duodenalis, 164
 Uncinariasis, 164
 Undetermined nitrogen of urine, 272
 Unilateral nephritis, 412
 Unit of counting chamber, 551
 Unna-Tänzer's stain, 12
 Unorganized sediments in urine, 374
 Unoxidized sulphur of the urine, 223
 Uranium method for phosphates, 218
 Urates in urine, 375
 Urea concentration test, 414
 Urea in blood, 501
 in urine, 241
 amount of, 242
 determination of, 242
 variations of, 242
 Urease method for urea, 247, 501
 Uremia, blood in, 504
 urine in, 406
 Uremic coefficient, 504
 constant, 504
 Ureometer of Doremus, 243
 of Hinds, 244
 Ureteral calculi, 402
 Ureteritis membranacea, 280
 Urethritis, anterior, 388
 posterior, 388
 Uric acid, 255, 508
 calculi, 403
 diathesis, 258
 in blood, 508
 in the urine, 255
 determination of, 259
 metabolism of, 256
 variations of, 257
 sediment, 374
 Uricacidemia, 514
 Urine, 188
 acetone in, 206, 354
 acidity of, 199
 albumin in, 289
 albumoses in, 305, 308
 alkaline tide of, 201
 alkapton bodies in, 196, 367
 alloxur bodies in, 206, 265, 376
 amino-acids in, 272
 ammonia in, 250
 amount of, 190
 animal gum in, 347
 parasites in, 402
 appearance of, 194
 ash of, 207
 bacteria in, 398
 Bence-Jones protein in, 305
 bile acids in, 365
 biliary pigments in, 363
 black, 197
 blood cells in, 389
 pigment in, 362
 blue, 197, 285
 calcium in, 229
 calculi in, 402
 carbohydrates in, 313
 carbonates in, 228, 384
 casts in, 391
 changes on standing of, 194
 chemistry of, 206
 chlorids in, 208
 cholesterin in, 381
 chromogens in, 281
 chyle in, 196, 381
 clearing of, 295, 333
 collection of, 189
 color of, 195
 composition of, 206
 consistence of, 194
 creatin in, 266, 271
 creatinin in, 266
 cryoscopy of, 407
 cultures of, 398
 cystin in, 225, 377, 405
 dextrin in, 347
 dextrose in, 313
 diacetic acid in, 358
 diastase in, 279
 drug reactions in, 196
 Ehrlich's benzaldehyde reaction in, 371
 diazo reaction in, 368
 egg-yellow reaction in, 369
 electric conductivity of, 408
 epithelial cells in, 384
 fat in, 381
 fatty acids in, 276
 ferments in, 279
 fibrin in, 312
 foreign bodies in, 402
 free mineral acidity of, 200
 organic acidity of, 200
 functional diagnosis from, 406
 glucose in, 314
 glycosuric acid in, 368
 glycuronic acid in, 347
 green, 196
 hematoporphyrin in, 362
 hemoglobin in, 310, 362
 hippuric acid in, 275, 380
 homogentisic acid in, 197, 367
 indican in, 284
 indigo in, 284
 inosite in, 347
 iron in, 231
 lactic acid in, 276
 lactose in, 345
 laidose in, 341
 leucin in, 272, 378
 leucocytes in, 386
 levulose in, 338
 magnesium in, 229
 maltose in, 346
 melanin in, 196, 366
 microscopy of, 371
 mucin-like substances in, 280
 mucoid material in, 384
 neutral sulphur in, 225
 nitrogen in, 231
 nitrogenous bodies in, 231
 nubecula in, 194, 395
 nuclein bodies in, 265
 nucleo-albumin in, 280
 odor of, 197
 optical activity of, 205
 organized sediments of, 384
 oxalic acid in, 277
 oxaluric acid in, 277

- Urine, β -oxybutyric acid in, 359
 parasites in, 402
 pentoses in, 341
 peptone in, 310
 phosphates in, 214, 381, 382
 physical properties of, 190
 pigments in, 281, 381
 potassium in, 228
 preservation of, 189
 protein of, 289
 proteoses in, 305
 ptomaines in, 377
 purin bases in, 265
 pus in, 386
 quantity of, 190
 reaction of, 198
 Russo's reaction in, 370
 sediments of, 374
 serum-albumin in, 289
 globulin in, 304
 skatoxyl in, 287
 sodium in, 228
 solids of, 204, 207
 specific gravity of, 202
 spermatozoa in, 398
 sugar in, 313
 sulphates in, 223
 sulphur compounds in, 223
 tissue fragments in, 398
 total solids of, 204, 207
 tyrosin in, 272, 379
 urates in, 375
 urea in, 241
 uric acid in, 255, 374
 urobilin in, 283
 urochrome in, 281
 uroerythrin in, 282
 urohematin in, 283
 urorosein in, 288
 xanthin bases in, 265, 376
- Urinod, 197
 Urinometer, 203
 Urinous odor, 197
 Urobilin, 283
 Urobilinuria, 283
 Urochrome, 281
 Urochromogen, Weisz' test for, 282
 Uroerythrin, 281
 Uroferric acid, 225
 Uroleucic acid, 206, 367
 Urophain, 288
 Urorhodin, 287
 Uroroseinogen, 288
 Urorubin, 287
 Urostealith calculi, 406
 Uterine secretions, 424
- Vaccination, antityphoid, 717, 877]
 Vaccines, 702, 873
 autogenous, 876
 diagnostic use of, 702
 preparation of, 874
 stock, 874
 Vaccine therapy, 702
 Vacuolization, 532
 Vaginal secretions, 421
 Vaginitis, catarrhal, 422
 gonorrhoeal, 423
 Value of blood examinations, 788
- Value of functional renal diagnosis, 406
 of search for tubercle bacilli, 23
 Van Deen's test for blood, 117, 784
 Van den Bergh's test for bilirubin in blood, 534
 Van Ermengem's stain for flagella, 847
 Van Gieson's stain for Negri bodies, 186
 Van Slyke's method for hemoglobin, 478
 for oxygen binding capacity, 478
 Van Slyke and Cullen's method for CO
 capacity of blood, 441
 for urea, 247, 501
 Van Slyke and, Donleavy's method for
 plasma chlorids, 537
 Van Slyke and Salvesen's method for
 carbon monoxid in blood, 473
 Van Slyke, Stillman and Cullen's titration
 method, 445
 Vaquez' disease, 590
 Variations in number of leucocytes, 607,
 618
 of red cells, 587, 595
 Variola, blood in, 652
 Venous blood, 435
 puncture, 434, 688, 727
 Vermiculus, 669
 Vernal conjunctivitis, 53
 Vesicular albuminuria, 290
 mole, 425
 Vibrion septique, 860
 Vincent's angina, 44
 bacillus, 44
 spirillum, 44
 Vinegar eel in urine, 402
 Viscosity of blood, 459
 Vitali's test for pus, 388
 Vital staining of blood cells, 579
 Voges-Proskauer reaction, 854
 Volatile alkalinity of urine, 202
 Volhard's method for chlorids, 211
 Volume index of blood, 433
 of blood, 429
 quotient, 433
 value, 433
- Vomitus, 66
 bile in, 67
 blood in, 67
 fecal, 67
 green, 67
 mucus in, 67
 odor of, 67
 pancreatic fluid in, 67
 parasites in, 67
 pus in, 67
 rice water, 67
- Wagner's test for blood, 119
 Wang's method for indican, 287
 Warfield's test for pregnancy, 273
 Warthin and Starry's method for spiro-
 nemata, 802
 Wassermann-fast types, 753
 Wassermann's serum reaction for syphilis,
 724
 Waxy casts, 303
 Weber's test for blood, 118
 Weidel's test for xanthin, 376
 Weil's disease, 683

- Weinstein's test for gastric carcinoma, 101
Weisz' test for urochromogen, 282
Welch's gas bacillus, 142, 858
Weyl's test for creatinin, 268
Whetstone crystals of uric acid, 374
 of xanthin, 376
Whip worm, 162
White blood cells (see Leucocytes), 597
Whitehorn's method for chlorids, 539
Whooping cough, bacillus of, 851
 blood in, 653
Widal reaction, 711
Williams and Lowden's stain for Negri
 bodies, 186
Williamson's blood test in diabetes, 645
Winternitz' method for gastric motility, 93
Winternitz', Henry and McPhedrans' test
 for catalase, 619
Wolff and Junghans' test, 102
Wright and Kinnicutt's method for blood
 plates, 563
Wright's opsonic method, 701
Wright's stain for blood smears, 576
 vaccine therapy, 702
Xanthin bases in feces, 131
 in urine, 206, 265, 376
 calculi, 405
Xanthochromasia, 813
Xerosis bacillus, 44
Xylose in urine, 341
Yaoita's method for ova, 143
Yeast cells in feces, 137
 in gastric contents, 69, 70
 in sputum, 16
 in urine, 325
Yellow fever, blood in, 681
 mosquito theory of, 681
Ziehl-Neelsen method for tubercle bacilli, 20
Zygotes, 669
Zymogens in gastric juice, 84, 88
Zymophore, 699

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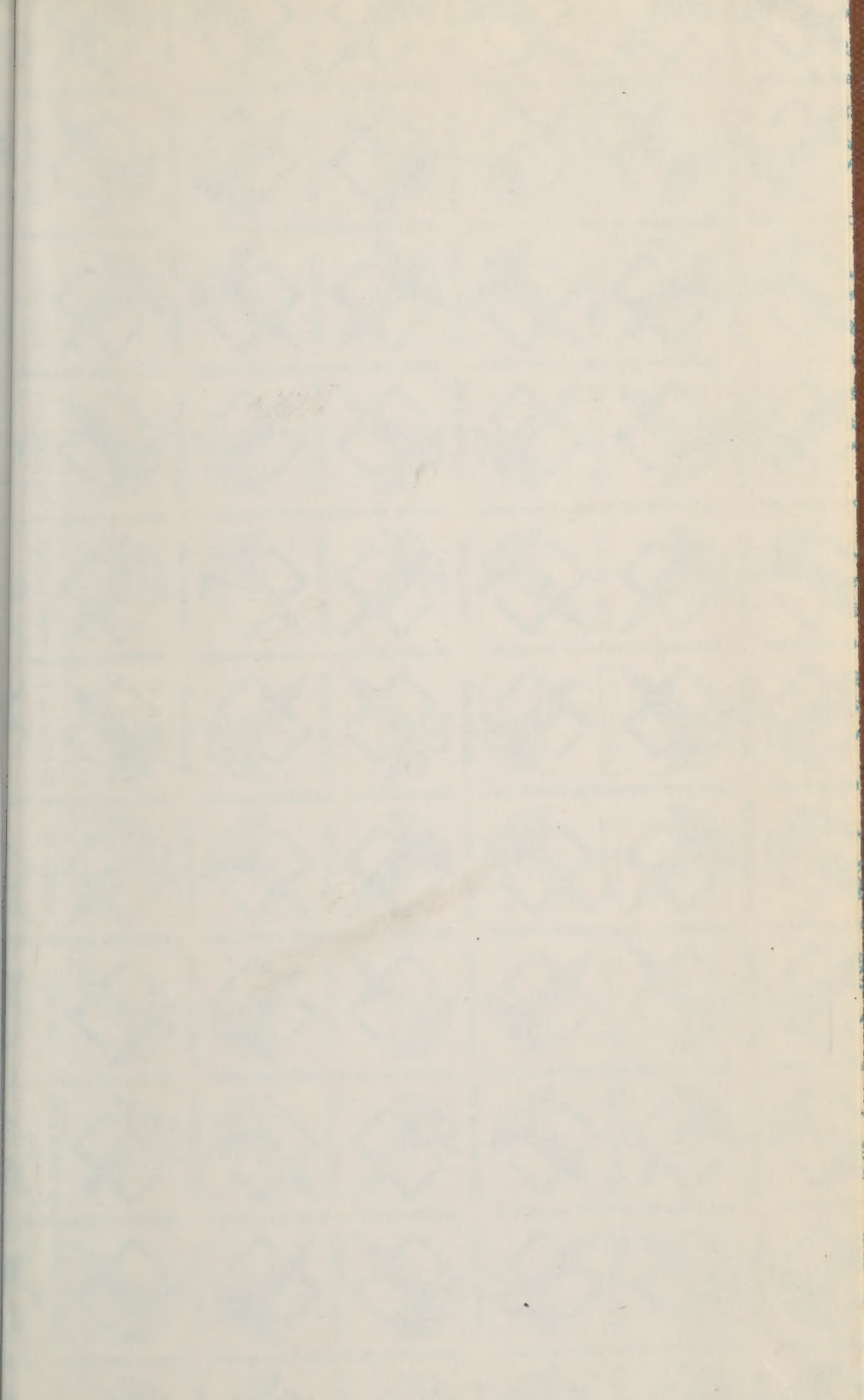
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